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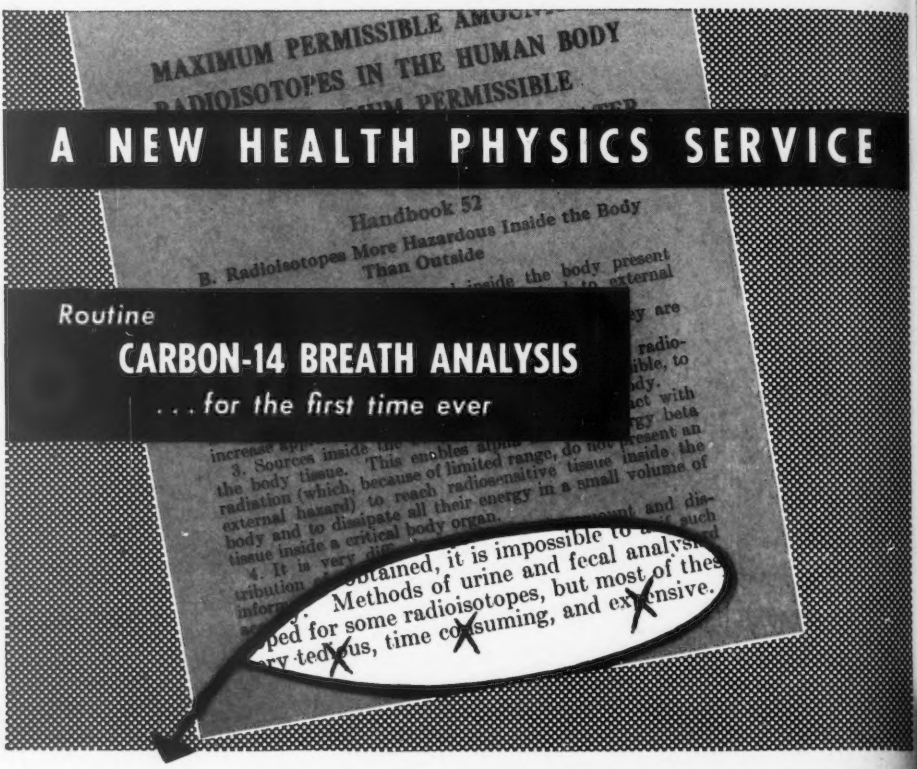

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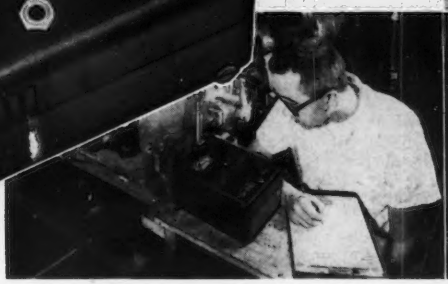

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President's Page

Nearly a decade ago, several officers and board members of our association participated in establishing within the American Association for the Advancement of Science, a new section of that association designated as Industrial Science (P). Affiliated with this section are the AIHA; the American Society of Safety Engineers; the Society for Industrial Microbiology; and the Southern Association of Science and Industry. The motivation for this development was the desire to provide for our members, through an AIHA sponsored channel, an opportunity to present the broad spectrum of industrial hygiene to other scientists not familiar with our profession. At the same time, it was felt that association with the many other societies affiliated with the AAAS would broaden the scope and horizons of our members.

Similarly, and to a greater degree, our membership and profession has benefited by participation in meetings of the Health Physics Society, the Acoustical Society of America, the Air Pollution Control Association, the American Society of Safety Engineers and many others. Last year, in response to the indicated need for coordination of AIHA efforts in joint undertakings with other professional societies, the Interprofessional Relations Committee was established. Initial representation on the committee included the fields of health physics, toxicology, safety engineering and medicine, as well as the general field of industrial hygiene.

Fundamentally, the recognition of the need for such a committee demonstrated the broad scope of interests of our members. One charge to the committee—i.e. to explore possible benefits to AIHA of continuing and improving existing relationships with other societies as well as establishing relations with additional organizations—was an indication of your board's concern that our horizons were too limited. Although the Interprofessional Relations Committee has not been involved in current developments concerning our annual meetings, the wisdom of appointing such a committee is becoming more obvious.

During the years, the strongest ties quite naturally have developed between the AIHA and the American Conference of Governmental Industrial Hygienists. Also, we have been fortunate, indeed, to be invited by the Industrial Medical Association to schedule our annual meetings at the time of and to participate in the annual "Industrial Health Conference". Certainly, there has been unquestioned mutual benefit to all five organizations comprising the conference. As the latter has grown, so has AIHA's share of the program and attendance of AIHA members. Similarly, responsibilities for management of the conference, including selection of meeting dates, cities and housing arrangements, establishment of registration fees, choice of exhibits managers and conference managers—responsibilities for these and many other financial details have increased to the point that the receipt, expenditure and commitment of your association's funds are important considerations.

Such considerations should not and will not take precedence over the more important aspects of the benefits derived from our association with IMA. However, proposed financing and accounting procedures accompanying an invitation to participate technically and professionally in an endeavor that has financial implications over which we have little or no control must be scrutinized. This does not represent a desire for independence; rather it appears to be an indication of mature business sense.

Members of the AIHA Board of Directors currently are studying an IMA proposal for financial management of future Industrial Health Conferences with the sincere hope that a plan may be developed which will allow your officers and board members to discharge their duties and responsibilities as your elected representatives in the successful management of the financial as well as other affairs of AIHA.

Elmer P. Wheeler

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TABLE OF CONTENTS

AMERICAN INDUSTRIAL HYGIENE ASSOCIATION JOURNAL, published bi-monthly for the American Industrial Hygiene Association. Dohrman H. Byers, *Editor;* Kenneth W. Nelson, *Associate Editor.* Editorial Offices, 1014 Broadway, Cincinnati 2, Ohio. Subscription $7.50 per year in the United States; $7.75 per year in Canada; $8.25 per year in all other foreign countries. Single copies $1.50. Entered as second class matter at the post office at Baltimore, Md. The American Industrial Hygiene Association Journal reserves the right to edit all advertisements and to refuse advertising copy when it does not meet the high standards adopted by the Association.

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An Approach to a Rational Method of Recommending Face Velocities for Laboratory Hoods*

J. E. PETERSON, M.S.

The Dow Chemical Company, Biochemical Research Laboratory, Midland, Michigan

THE BASIC function of exhaust hoods is to control contaminated air in such a manner that the contaminant does not reach the breathing zone of the user in significant quantities. Laboratory hoods differ from many other types in that the contaminant is released and becomes airborne entirely within the structure, and the problem then becomes one of keeping the contaminant inside the hood until it is discharged through the exhaust system. Since the main route of contaminated air escape to the breathing zone of hood-users is normally through the hood face, performance of a laboratory hood is a function of the behavior of air at the face opening. Therefore, a single number, the mean (average) face velocity it the usual criterion employed, and under the proper circumstances it is a complete specification of laboratory hood performance.

Laboratory exhaust hoods vary tremendously in design, but are represented by three main types: the common bench hood, the double-face lattice hood, and the walk-in hood. All types may be purchased complete from hood manufacturers, or may be constructed on the spot; they may or may not have an internal or external baffling system, and they may or may not be equipped with doors or sashes. Open face areas may vary from two to three square feet to 100 square feet or more. Hoods may be located in a quiet corner of a laboratory or occasionally, they may be found installed next to an open door or window, or in a location where foot traffic approaches that of Main Street. Chemicals handled in laboratory hoods may range in toxicity from water to hydrogen cyanide to nickel carbonyl.

In the face of this variety, one often finds in the literature that a single figure is specified as being *the one* to attain insofar as face velocity is concerned. Less often a range of values will be mentioned which may vary with the toxicity of chemicals handled within the structure, or may depend upon whether the hood is to be used by high school students or by industrial chemists. The values recommended as being adequate face velocities range from 40 fpm (lineal feet per minute) to 200 fpm or more.

This situation was decried by Schulte and coworkers[1] who, in 1954, published the results of their experiments with bench hoods. In recommending 100 fpm for an average face velocity they specified that the chemicals handled should be of moderate toxicity, that the hood should be located away from sources of air disturbance, that the hood entrance should be kept free of obstructions and that the minimum air velocity at the face should be 80 fpm. They showed that even relatively high heat loadings within this type of hood had little effect on performance.

Later, W. B. Harris went even farther in Sax's *Dangerous Properties of Industrial Materials*.[2] In the "Ventilation Control" section he, by means of a number of tables, related recommended face velocity to local air motion, to the fire or toxic hazard, and to the "volatility" (vapor pressure in mm of Hg at 20°C) of the material handled.

However, in most practice, face velocities for laboratory hoods have been recommended on the basis of judgement coupled with experience. Such judgement has usually been made after consideration of a number of variables, with little or no thought being given to the weight or importance of each or, in fact, of which variables to consider at all. Consequently, a method was sought which would force consideration of each variable separately and which would combine the judgements so made into a defensible and rationally determined recommended face velocity specific for each hood.

The first step in the development of a new technique for specifying adequate face velocities for laboratory hoods was to list the variables involved. The most important of these appeared to be as follows:

1. Toxicity of the material(s) to be handled within the hood.
2. Volatility of the material(s) to be handled within the hood.
3. Effects of air disturbance created outside the hood.
4. Effects of air disturbance created within the hood.

* Presented at the Twentieth Annual Meeting of the American Industrial Hygiene Association, April 25–May 1, 1959, Chicago, Illinois.

5. Characteristics of the hood.
6. Variability of air flow at the face of the hood.

Concurrently, a search of the literature was made for recommended face velocities for laboratory hoods.[1, 3-14] This search revealed that, while confusion seemed to predominate, some order did exist. The range of recommended face velocities for materials of low toxicity began at about 50 fpm and extended upwards. For materials of high toxicity, the range began near 100 fpm.

In order to use these figures in a new technique, an assumption was made that values at the low end of each range were intended to apply to the best hoods, while higher face velocities would be necessary for those not quite so efficient.

Thus was initiated the concept of a "standard hood" for which the effect of all variables except those related to the material handled would be negligible. Wide experience reported in the literature in the form of face velocities could therefore be utilized; a face velocity of 50 fpm would be assigned for handling materials of least hazard in the standard hood, and 100 fpm for those of most hazard, with others falling between these extremes. Face velocity values so determined would then be modified as necessary by values assigned to the other variables in proportion to their deviation from the specifications of the standard hood.

In accordance with this concept, the several variables were combined into three factors: a Vapor Control Factor dependent upon characteristics of the materials handled, an Environment Factor proportional to expected air disturbance, and a Hood Characteristic Factor proportional to the size and shape of the face opening. These factors were arranged into the following equation:

$$V_r = M\ (1.0 + E + C) \qquad (1)$$

where:

Vr = Recommended Face Velocity
M = Vapor Control Factor
E = Environment Factor
C = Hood Characteristic Factor.

This equation was complete except that variability of air flow at the face of the hood was yet to be considered. In determining the mean face velocity of a laboratory hood a number of measurements are made and averaged. Since these items of information are available, their standard deviation can be calculated, and it is an excellent measure of air flow variability. In order to apply this concept, an assumption was made that the minimum air velocity at a hood face could probably be as low as 75 per cent of the mean face velocity, as calculated in Equation (1), without affecting hood performance appreciably. If, to this minimum, twice the standard deviation were added, a new mean face velocity would result which, if adopted as the recommended mean face velocity, would assure that 98 per cent of the individual values would be above the minimum.

In practice, the statistic most useful for comparing variability of air flows was found to be the variance or square of the standard deviation. Therefore, that statistic was used in the following equation.

$$V_r = 0.75\mathrm{M}(1.0 + E + C) + 2\sqrt{s_x^2} \qquad (2)$$

where: s_x^2 = the variance of individual measurements.

Equation (2) was the final form of the equation used to calculate recommended face velocities. After its establishment, techniques were necessary for assigning numerical values to each of the included factors in a more or less standard manner.

Vapor Control Factor, M

As previously stated, many authors have recommended face velocities for laboratory hoods which varied according to the properties of the material handled, mainly the toxicity and/or the volatility. Needed then, was some way of associating these properties with the Vapor Control Factor.

For many years industrial hygienists have been in the business of assuring that vapor concentrations of materials are kept at or below certain levels. These levels have been given many names, but all fall under the generic term of "control concentrations," and have been arrived at by consideration of comfort as well as toxicity, and by an estimation of acute as well as chronic effects. While these values are not proportional to toxicity, they are estimations of the degree of control desirable in a work environment. And, as such, they were felt to be quite suitable as parameters of the Vapor Control Factor. The actual value used for any particular material as the control concentration might be arrived at as the result of experience, from a study of original toxicological data, or by consulting the various compilations of such values published by the American Conference of Governmental Hygienists, the American Standards Association, the American Industrial Hygiene Association, or regulatory bodies such as state agencies.

Several factors are combined in the general term "volatility." The volatility of materials de-

pends upon temperature, molecular weight, surface tension, latent heat of vaporization, vapor pressure, surface area, etc. To incorporate all of these variables into a volatility index would make it so cumbersome as to be useless, especially since values for each of these variables are not obtainable for many materials.

Evaporation rate was felt to approach what is meant by volatility rather closely, and evaporation rate data are available for a fairly large number of materials. However, evaporation rate data have several shortcomings.[15] A number of compilations of these data were found,[16, 17] none of which was directly relatable to any other. Nearly always, evaporation rates were found to have been determined under a set of specially restricted conditions which applied with great utility to those conditions, but with limited utility to others. For example, evaporation rates are usually associated with a single temperature, while materials handled in hoods are subject to all temperature extremes. And also, such data are usually available only for solvents. For these reasons, the use of published evaporation rate data as an index of volatility was abandoned.

Vapor pressure was next considered. Obvious advantages of using this variable as an index of volatility were: such data are freely available for most materials; vapor pressure is a physical property, and the conditions of measurement are immaterial and each list of data is directly comparable to all others; vapor pressures are published as a function of temperature; and, vapor pressure is an obviously large component of volatility. The obvious disadvantage is that vapor pressure is only one component of volatility.

In the *Handbook of Solvents* by Scheflan and Jacobs[18] the following statement was found: "One method of calculating the relative rate of evaporation of solvents approximately is to multiply the molecular weight of the solvent by its vapor pressure at the temperature for which an estimation of the rate is desired." Here, then, appeared to be a solution to the problem of a volatility index. Scheflan and Jacobs cautioned that their statement did not always hold true, but for the Vapor Control Factor, their technique appeared to be a good compromise between using either published evaporation rates or vapor pressures—neither of which was suitable.

Another consideration associated with the Vapor Control Factor was the quantity of material handled. Rather than introduce this as another variable a decision was made to simply limit the amount of material to which this technique would apply. Observation and consultation revealed that hoods are normally used for handling up to a few hundred grams of most materials without special precautions. This technique, therefore, was restricted to apply to the handling of a "few hundred grams or less" of materials for which hoods are normally used.

The Vapor Control Factor was then considered to be directly proportional to the vapor pressure times molecular weight, and inversely proportional to the control concentration. For about 170 materials, vapor pressures at room temperature were found and expressed in millimeters of mercury (where the material was a gas, 760 mm of Hg was used). These vapor pressures were multiplied by the molecular weight of each material and divided by the control concentration, expressed in ppm, to form a ratio, R. The materials considered were then arranged on a list in sequence of ascending R values which ranged from 0.06 (phenyl glycidyl ether) to 65,600,000 (nickel carbonyl).

Examination of the sequential list revealed that all materials with an R of over 50,000 appeared to warrant the highest Vapor Control Factor, namely 100, while those with an R of 3.0 or less appeared to be materials which normally were safely handled outside of a hood in laboratory quantities.

At this point, either a table containing appropriate Vapor Control Factors for R value groupings or a graph containing similar information could have been used. Personal preference dictated the use of a graph. Because of the wide spread in magnitude, a logarithmic scale for the R values was chosen, while an arithmetic scale seemed appropriate for the Vapor Control Factors.

By previously assigning Vapor Control Factors at both ends of the R scale, the shape of the curve was fixed. It would be S-shaped, approaching a Vapor Control Factor of 100 near an R value of 50,000, and approaching a Vapor Control Factor of 50 near an R of 3.0. Carbon tetrachloride with an R of about 755 at room temperature and benzene with a value of 297 seemed to be representative of those materials deserving an intermediate Vapor Control Factor of about 75. A curve was then drawn to conform to these ideas, and appears in Figure 1.

Environment Factor, E

When the variance (or standard deviation) is calculated, the effect of air disturbance during the hood evaluation is accounted for when the recommended face velocity is calculated. In many cases for existing hoods, and in all cases for hoods which are still on the drawing board, some sources of air disturbance will not be operating during hood evaluation. This is exemplified by the prob-

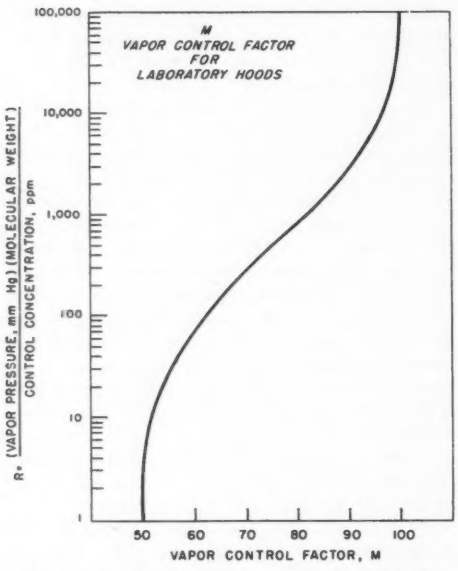

FIGURE 1. Vapor Control Factor for use in Equation (2).

lem of intermittent foot traffic, or that of a draft from an adjacent window. In such cases, the usual solution is to eliminate as much air disturbance as possible while finding face velocities, and to try to compensate for it later when determining the air flow necessary for that hood. The Environment Factor was the result of an attempt to systematize these compensations.

The "standard hood" was, of course, used as the starting point. It was specified to be in a location devoid of foot traffic and drafts, where the velocity of disturbing air currents was 25 fpm or less. Also, the hood was specified to contain no equipment which would cause a disturbance in the flow of air entering at its face.

Limited experimental work especially by Schulte and co-workers[1] has shown the great effect caused by supposedly minor sources of air disturbance such as persons passing by the hood in question. They found "... that whenever the velocity of the cross currents equals or exceeds the face velocity, serious disturbance of the hood air flow pattern results."

A good deal more experimental work is necessary before the effects of cross currents on air flow patterns will be completely understood. Until such work is done, numerical values given to the Environment Factor must be drawn from experience and must be applied with due consideration given to their source. The guide in Table I has been drawn from work done with a relatively large number of laboratory hoods. It has given results which appear to be practical.

Approximate values of the velocity of the disturbing air appropriate to these classifications were tentatively assigned, partly from measurement and partly from experience. Accordingly, Figure 2 which relates the Environment Factor to

TABLE I
Environment Factor Guide

Classification of air disturbance	E factor
1. Minor air disturbance. The hood is located away from foot traffic, drafts from open doors or windows, air circulating fans, etc.	0 to 0.15
2. Moderate air disturbance. The hood is located where persons occasionally walk by, near an inside door which is usually closed, etc.	0.15 to 0.45
3. Severe air disturbance. The hood is located where persons frequently pass, near an inside door usually open, etc.	0.45 to 0.75
4. Very severe air disturbance. The hood is located near an outside door or window which may be opened; air from a fan or heater impinges on the hood face, etc.	0.75 and up

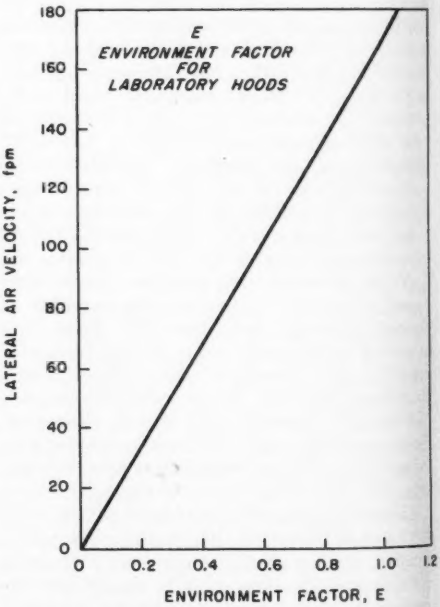

FIGURE 2. Environment Factor for use in Equation (2). The width and slope of this curve are somewhat questionable; use with discretion.

the velocity of lateral air flow was drawn. The curve should be regarded as being a band of somewhat questionable width and slope rather than as a firmly fixed line. As more quantitative experience is gained a better evaluation of this factor should be possible.

Hood Characteristic Factor, C

Some years ago Dalla Valle[19] showed that the ability of an exhaust hood to reach out and capture air is much more a function of the volume of air moved through the hood than it is of the velocity of that air at the hood face. These findings are implicit in two equations of interest, one for flanged hoods and one for unflanged hoods. Rearranging his equation for a flanged hood:

$$V_0 = \frac{V(X^{1.5} + c'A^{0.82})}{c'A^{0.82}} \qquad (3)$$

where:

V_0 = the velocity of air at the face of the hood, fpm.
V = the velocity of air at the centerline, X feet from the hood face, fpm.
X = the centerline distance from the face of the hood to the point of interest, feet.
A = the face area of the hood, square feet.
c' = a variable constant, proportional to the ratio of the width to the length of the face opening.

Similarly, for an unflanged hood:

$$V_0 = \frac{V(X^{1.91} + bA^{1.04})}{bA^{1.04}} \qquad (4)$$

where:

b = a variable constant similar to c′ but with different values.

In working with these equations it soon became apparent that Dalla Valle had done most of his work with hoods having smaller face openings than those usually associated with laboratory hoods, as was to be expected since his main interest was not with large hoods. Above an area of about 10 square feet his equations indicated that under similar circumstances an unflanged hood was more efficient than a flanged hood. Nevertheless, these two equations were used to investigate the recapture efficiency of laboratory exhaust hoods. In the area of discrepancy the equation for unflanged hoods was used for both types.

Laboratory hoods are not normally equipped with true external baffles (flanges). However, many of the newer hoods are constructed with picture frame or air flow (or foil) baffles external to the face. Experiment has shown that such a system is comparable or superior in efficiency to a flat flange-type baffle, and therefore, Dalla Valle's equation for externally baffled hoods was assumed to apply.

Basic to the use of these equations and to the calculation of a Hood Characteristic Factor was the premise that all laboratory hoods regardless of size should offer equivalent recapture protection. Large hoods through which relatively large volumes of air move do offer more protection than smaller hoods with the same face velocity. Therefore, instead of penalizing the larger hoods by insisting on face velocities high enough to be adequate for the smaller ones, an attempt was made to equalize the protection given with a Hood Characteristic Factor. This concept has been used in specifying face velocities for paint spray booths.[20, 21] The same consideration of equality of protection applies to the shape of the hood face and to the presence or absence of external baffles; variables present in the Dalla Valle equations.

In order to utilize these equations to calculate the effect of deviations from the "standard hood," specifications for that structure were written. The most common, and also generally the smallest of the hood types is the bench hood. This type was therefore chosen for the "standard hood." A survey of over 100 bench hoods, both purchased and homemade, revealed that the average hood had an open face area of 11.1 square feet. To use round numbers, 10.0 square feet was chosen for the "standard hood." The most efficient hood for recapture purposes was shown by Dalla Valle's work to have a flanged, square face opening, and such was specified for the "standard hood."

Using Equations (3) and (4), the graphs shown in Figures 3 and 4 were constructed relating C, the Hood Characteristic Factor to the face area and side ratio for flanged and unflanged hoods. These curves were based on the assumption that all hoods should have control of vapors one foot from the face equivalent to that achieved by the "standard hood."

Variance of Face Velocity Data, s_z^2

Variance is a statistical term, the square of the standard deviation of individual measurements. It is calculated one of two ways, depending mainly on whether the work is done by hand or by a machine calculator. (Any standard book on statistics may be consulted for further information.) The two techniques are equivalent; either may be used.

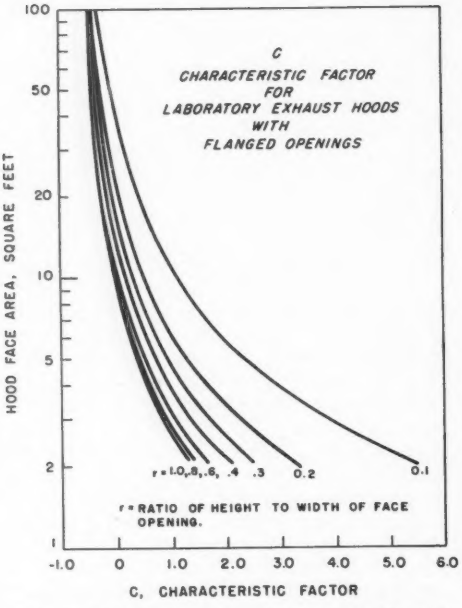

FIGURE 3. Characteristic Factor for hoods with flanged openings. From Dalla Valle's equation, to provide equivalent air velocity one foot from the hood face.

Most convenient by hand:

$$s_x^2 = \frac{\Sigma(X - \bar{X})^2}{n - 1}$$

where:

s_x^2 = the variance of individual measurements
X = an individual measurement
$\bar{X}$ = arithmetic mean of the measurements
n = the number of measurements
Σ = the symbol indicating summation

Most convenient by machine:

$$s_x^2 = \frac{\Sigma X^2 - [(\Sigma X)^2/n]}{n - 1}$$

Experience with a large number of laboratory hoods has given some idea of the range of variances which may be encountered. Such experience is valuable when using this method of calculating recommended face velocities for hoods to be constructed or installed. In cases where the external air disturbance was negligible, the values of the variance shown in Table II were found. The hoods from which these data were gathered were in use; none were overcrowded, but all contained some laboratory equipment.

Any of the variances in Table II could easily be doubled or tripled by internal air disturbance factors such as air stirrers, rapidly moving equipment, high thermal loads, or by poor placement and/or crowding of equipment within the hood.

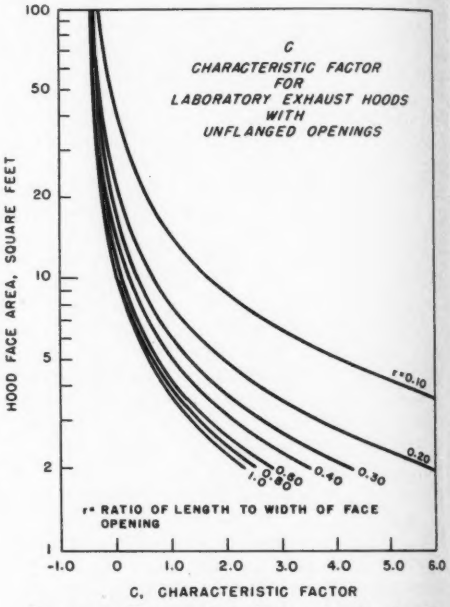

FIGURE 4. Characteristic Factor for hoods with unflanged openings. From Dalla Valle's equation, to provide equivalent air velocity one foot from the hood face.

TABLE II
Variance Summary

Hood type	Average variance	Variance range	Number of hoods
Bench			
Internal and external baffles	130	26 to 265	35
Internal baffles only	217	26 to 756	58
No baffles	714	138 to 2812	17
Lattice			
Internal and external baffles	Insufficient data		
Internal baffles only	Insufficient data		
No baffles	358	61 to 687	9
Walk-in			
Internal and external baffles	141	27 to 246	9
Internal baffle only	Insufficient data		
No baffles	Insufficient data		

Similarly, external air disturbances may cause the variance to be large. A variance of 15,600 was the highest encountered in a survey of about 200 hoods; it was caused by the impingement of air from a unit heater directly on a hood face.

Discussion

The equation for calculating recommended face velocities for laboratory hoods has been tested by experience and found to be practical. Since its development the values assigned to each of the factors have been modified several times when such modification was found to be desirable. Recommended face velocities for existing and for proposed hoods have been calculated and put into practice at The Dow Chemical Company with good results. In actual use, the values calculated are rounded, usually to the nearest 5 fpm, and further rounding is usually practiced when specifying volumetric flow rates.

The type of analysis presented has several advantages. It forces the user to examine separately each of the factors influencing hood performance. These factors may not be independent, but each is treated as if it were, and the contribution of each factor may be analyzed. In cases where excessively high recommended face velocities are calculated, changing the hood or its environment might well be more practical than insisting on a face velocity adequate to contain air within the hood under the existing conditions.

Also, use of a technique such as has been proposed might tend to somewhat standardize recommended face velocities. This technique has been used extensively for about a year by three individuals with widely varying backgrounds in The Dow Chemical Company's Biochemical Research Laboratory, Environmental Research Section. In many cases all three have made recommendations for the same hood with surprisingly consistent results.

In all, face velocity recommendations have been made with this method for over 200 existing laboratory hoods, including bench, lattice and walk-in types. Judged by the degree of satisfaction of users, it has been successful. Data obtained on the hoods studied have been used in modifying the technique, and have also been used to make possible face velocity recommendations for over 60 hoods in the design stage. Therefore, the basis of a rational approach to this problem has been established and has been shown to result in satisfactory face velocity recommendations.

Future refinements may well change the method of assigning numerical values to any or all of the factors and may even change the form of the equation, but the technique, even in its present state, should prove to be useful to many industrial hygiene engineers. Such engineers, however, should keep in mind that in using this method they are not manipulating mathmatical entities, but are merely calculating judgements, and judgements must still be based on experience.

Summary

Variables affecting the performance of laboratory hoods have been combined into an equation for the calculation of specific recommended face velocities for each hood. The equation is strictly empirical and numerical values are assigned to each of the factors on the basis of judgement coupled with techniques developed for that purpose.

In use, the Vapor Control Factor is modified by an Environment Factor and a Hood Characteristic Factor to achieve a face velocity which would be recommended for a "standard hood." Then, a statistic (the variance) is utilized to calculate a mean face velocity which will assure that 98 per cent of the individual velocity measurements at the hood face will fall above a minimum, calculated as 75 per cent of the mean face velocity for the "standard hood."

Specifications of the "standard hood" are discussed, as is the technique of assigning numerical values to each of the factors in the equation. Values of the variance are determined by measurement, or are estimated from an included table of variances associated with hood types.

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WESTERN INDUSTRIAL HEALTH CONFERENCE

THE THIRD ANNUAL Western Industrial Health Conference will be held October 2–3, 1959, at the Statler-Hilton Hotel in Los Angeles, California. On the first day there will be three sessions devoted to discussions of the following topics: *Psychologic Stresses, Practical Medical Management of Cardiacs in Industry,* and *Air Pollution and Health.* Specialty meetings of various professional groups will be held the morning of the second day. In the afternoon a panel discussion of *Industrial Health in the Space Age* will be followed by a movie, *Launching and Flight of an I.C.B.M.* The conference will be closed with a banquet on Saturday evening. At the time we go to press complete data on the final program is not available; for this information address L. C. Chase, Loss Prevention Department, Liberty Mutual Insurance Company, 714 South Hill Street, Los Angeles 14, California.

INSTITUTE OF INDUSTRIAL MEDICINE

THE NEW YORK UNIVERSITY Post-Graduate Medical School Institute of Industrial Medicine will offer a two month course in Occupational Medicine September 14 through November 6, 1959. The course is designed for physicians engaged in the practice of medicine in industry, full-time or part-time. Didactic instruction will be supplemented with field trips to industrial plants, to governmental agencies, and to union health centers. Participants will be given opportunity to attend medical, surgical, and clinical-pathology conferences at the New York University-Bellevue Medical Center.

Instruction will be divided into four broad areas: Preventive Medicine, Administrative Medicine, Occupational Diseases, and Industrial Hygiene. Each of these areas will be presented in detail with emphasis on the physician's understanding, participation, and responsibilities in the practice of occupational medicine and prevention of occupational disease.

For complete information address Office of Associate Dean, New York University Post-Graduate Medical School, 550 First Avenue, New York 16, New York.

A Granular Collector for Sampling Fallout Debris from Nuclear Detonations*

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Introduction

SEVERAL DIFFERENT kinds of sampling devices have been used to collect fallout debris from nuclear detonations and from certain types of nuclear reactors. These sampling devices fall into three general categories: (1) adhesive surfaces, (2) open containers, (3) air samplers equipped with filters, Cascade impactors, or electrostatic precipitators. Although each device has certain advantages, none has proved to be completely satisfactory for collecting fallout debris in amounts adequate to study the physical and chemical properties of fallout particles and the biological availability of fission products present in fallout.

The different kinds of sampling devices listed above have been used by this laboratory for collecting fallout debris in the natural environs adjacent to the Trinity and Nevada Test Sites.[1-8] Samples of native soil also have been collected from fallout areas and analyzed to supply data on area contamination and particle size distribution relative to distance from Ground Zero. These data have been used to delineate fallout patterns and to compare the actual deposition of fallout with that which had been predicted. Although the soil surface might be the ideal collector for measuring fallout deposition in the natural environment, it has three disadvantages: (1) there is a tremendous dilution of the radioactive fallout particles with inert soil, (2) there is always the possibility that soil samples contaminated with recent fallout might have received previous contamination, (3) considerable analytical difficulty is encountered in the physical and chemical characterization of fallout particles mixed with large amounts of soil.

Adhesive surfaces might not give representative measurement of fallout debris deposited in the natural environment because they do not always retain particles of different size ranges with equal efficiency.[9] Furthermore, the reclamation of fallout debris from adhesive surfaces is not practical in terms of the processing time and the hazards involved in using the large volumes of toxic solvents necessary to separate fallout particles from the adhesives. Open containers are not always efficient collectors because wind action may remove or selectively redistribute fallout debris of certain particle size ranges depending upon the wind velocities encountered during the sampling period. Air samples generally measure cumulative amounts of airborne particles which are difficult to compare to the unit area contamination from fallout deposited on the soil surface. Also, the expense of air sampling devices prohibits sufficient coverage of large areas of fallout contamination such as those encountered during nuclear weapons testing series.

In order to overcome some of the limitations of the conventional fallout collectors previously used, a granular collector was developed which permits the recovery of essentially pure fallout debris. This collector was used extensively during the 1957 Weapons Testing Series in areas adjacent to the Nevada Test Site in conjunction with Program 37, Civil Effects Test Group.

Materials and Methods

The collector support consists of a 29 x 43 x ⅝-inch deep metal tray (Boyco Auto Drip Pan, U. S. Steel Corporation) which is divided by taping a ½ x ½ x 28-inch wooden bar to the center of the tray. Each half of the tray is lined with 0.5 mil thick mylar film (Du Pont Corporation) and contains approximately 1.5 kg of polyethylene plastic granules (Figure 1). The matrix found to be most suitable was smooth, spherical, natural polyethylene pellets, ⅛- to 3/16-inch diameter, free from lint, fine dust, gels or soap-base products ("Tenite", produced by Tennessee Eastman Company, Kingsport, Tennessee). Washed pea gravel, quartz sand, glass beads, and several granular forms of synthetic materials

* This work was conducted under Contract No. AT(04-1)-GEN-12, between the Department and Laboratories of Nuclear Medicine and Radiation Biology, School of Medicine, University of California at Los Angeles, and the U. S. Atomic Energy Commission.

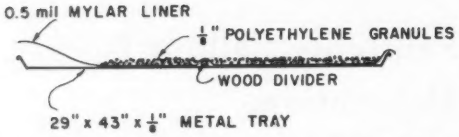

FIGURE 1. Cross-section of the granular fallout collector.

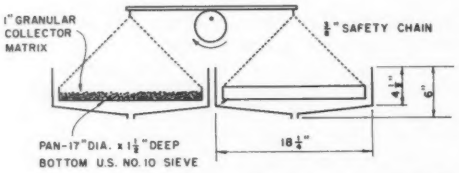

FIGURE 2. Cross-section of the wet sieve particle separator accomodating two removable 17-inch diameter × 1½-inch deep U. S. No. 10 sieves filled to 1-inch depth with polyethylene granules.

were tested but they were found to be less suitable than the polyethylene pellets.

A wet sieving process for separating the entrapped fallout debris from the granular collector matrix is necessary for maximum separation efficiency and to eliminate dust and attendant health hazards in handling the radioactive material during the recovery operation. This requires the use of a non-polar solvent that will assure a minimum solubility of the fallout debris. Isopropyl alcohol was selected for the separator solvent because it is relatively inexpensive, readily available in large quantities, non-toxic when handling large quantities under fume-hood conditions, and relatively easy to reclaim. Also, it does not decompose the Millipore filter disks required for efficient filtering of fine particles.

The wet sieve particle separator consists of two, 17-inch diameter, mechanically-driven U. S. No. 10 sieves which accomodate both halves of a granular collector tray sample for the separation of particles less than 2-mm diameter (Figure 2). A cradle supporting the sieves is activated by a revolving cam designed to lower and raise the sieves 28.5 times per minute. The wet sieving is done in sieving pans 18¾ inches in diameter, using 4.5 liters of isopropyl alcohol which covers the granular matrix when the sieve is in the lowered position. Upward displacement is sufficient to raise the sieve clear of the alcohol. The size of the sieve was selected to accomodate 1.5 kg of the plastic granules. A funnel in the bottom of the sieve pan facilitates transfer of the alcohol and separated fallout particles.

Particles greater than 44 microns in diameter are fractionated into 13 different size ranges in a dry sieve assembly (Figure 3). Removable screens of sieve sizes listed in Table I, (Unique Wire Weaving Company, Inc., Hillside, New Jersey) are mounted between aluminum spacer rings and inserted into a brass jacket which holds the screens firmly in the spacer rings while the assembly is being shaken on a Ro-Tap shaker. The jacket also traps radioactive dust which might arise during the dry sieving process. A metal rim around each screen holds them flat during radio-assay and sample storage. (The 1,000 μ screen is sufficiently rigid to remain flat

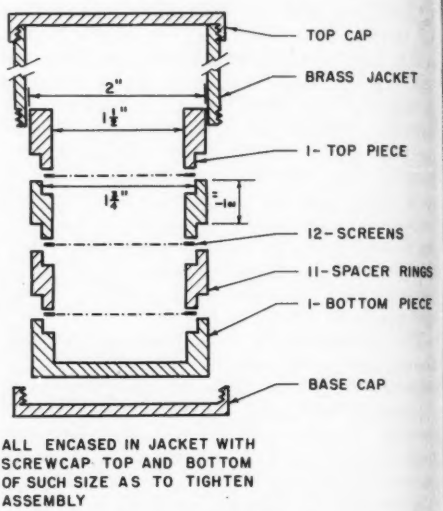

FIGURE 3. Cross-section of the dry sieve assembly for separating fallout debris into different particle size fractions.

TABLE I

Removable Screens Used for Sizing Fallout Particles

U. S. Sieve No.	Sieve opening (microns)	Mesh per cm	Mesh per inch
18	1,000	7	17.2
35	500	13	32.3
45	350	18	44.7
50	300	20	52.4
60	250	24	61.7
70	210	29	72.5
80	177	34	85.5
100	149	40	101
120	125	47	120
140	105	56	143
170	88	66	167
325	44	125	323

without a rim.) The sieve size ranges selected are those which have been used previously for fractionating the fallout contaminated soils collected from the environs of the Nevada Test Site.[1, 2, 5]

Particles less than 44 microns in diameter are separated from the isopropyl alcohol by vacuum filtration on a Millipore filter disk mounted in a Pyrex filter holder (Millipore Corporation, Watertown, Massachusetts, Cat. #XX 10-07-00). In assembling the unit, a 5.5-cm Whatman No. 1 filter disk is placed under a 47-mm type HA Milliport filter disk to cushion it from the fritted glass filter base. A 1/16-inch thick vellumoid gasket (1½-inch inside diameter, 1⅞-inch outside diameter) is centered on top of the Milliport filter disk. Upon clamping the apparatus together with the spring clamp, the vellumoid gasket raises the filter holder 1/16-inch above the Millipore filter disk and prevents the filtered particles from adhering to the base of the reservoir. It also limits the filtering surface to the same area as the removable screen in the dry sieving apparatus. After filtering, and before the filter holder assembly is dismantled, the apparatus is dried under a heat lamp for four minutes. This heating and drying process causes the alcohol-wetted Millipore filter disk to stick to the vellumoid gasket, which forms a taut, flat surface on which particles remain during radioassay and storage of samples.

The counting cup is a 50 mm, plastic culture dish suitable for the radio-assay and storage of the fractionated fallout particles and the gasket mounted Milliport filter disks (Leslie R. Burt, Ltd., 1524A South Baldwin Avenue, Arcadia, California). The samples are counted against Sr^{90} standards mounted in the plastic counting cups in such a manner that backscatter characteristics are comparable for both the standards and the samples. The use of Sr^{90} as a reference standard for fallout materials originally was decided by committee action in August, 1952, because it represents a fair energy spectrum of the beta particles emitted from fission products. This committee included members from New York Operations Office, Los Alamos Scientific Laboratory, U.C.L.A. Atomic Energy Project, and the Division of Biology and Medicine, Atomic Energy Commission.

Operational Procedure

During weapons testing operations where detonations may occur at close intervals, all sampling equipment must be protected from contamination during transport through areas previously contaminated with fallout. For this reason, pre-fabricated components of the fallout collector are transported to the sampling locations in separate, packaged units where they are assembled and placed on the ground in an unobstructed area. This operation generally takes place during the 12-hour period preceding a nuclear detonation. After fallout has occurred and the radioactivity in the area has diminished sufficiently to permit recovery (H + 24 hrs), the granular collector matrix is carefully removed from the metal tray by gathering together the sides and corners of the mylar liners to form bags which are fastened securely with paper-wrapped wire. The bags are returned to a shipping container for transportation to the processing laboratory.

Primary fallout particles are separated from the granular matrix by the following procedure which is accomplished in fume hoods in the laboratory:

1. Approximately 500 ml of isopropyl alcohol is added to the collector matrix in the bag formed from the mylar liner, and the wet collector matrix is transferred to the wet sieving apparatus. The liner is clipped to a stainless steel drain tray and any fine particles are removed with a rubber squeegee under a jet stream of isopropyl alcohol. During the process of washing and rinsing, four liters of alcohol are added to the wet sieve apparatus. Two halves of a collector tray sample prepared in this manner are wet sieved mechanically for five minutes (Figure 4). Following the wet sieving action, the screens are raised clear of the liquid and sprayed with one liter of fresh alcohol under pressure to displace the liquid entrapped in the matrix. The alcohol and separated particles are drained and rinsed through a bottom outlet in the sieve pan onto a 3-inch diameter U. S. No. 325 sieve (44 μ) supported in a funnel that delivers the alcoholic

FIGURE 4. Fallout separation from the granular matrix by wet sieving.

suspension of the less than 44-micron diameter particles into a 6-liter Bain-Marie enameled pot.

2. The alcoholic suspension of less than 44-micron diameter particles is passed through the Millipore filter apparatus under vacuum. Three rinses are used to remove all particulate material from the enameled pot. After the sample is dried under infra-red lamps, the Millipore filter disk holding the less than 44-micron particles is removed and placed in the plastic counting cup ready for radio-assay. Selected samples of the alcoholic suspension of less than 44-micron particles may be further fractionated into smaller size ranges by sedimentation techniques.[3]

3. The spacer rings and 12 removable screens in the dry sieve assembly are mounted on the base cap. The greater than 44-micron diameter particles are quantitatively transferred from the No. 325 sieve onto the top screen and the jacket is screwed into the base cap so that the spacer rings and removable screens are held firmly together during the dry sieving operation. It is mandatory that all component parts of this assembly be washed in acetone preceding each use to keep them free from moisture or greasy substances which cause particles to stick to the metal surfaces.

4. Four dry-sieve assemblies, each containing separate samples, are placed between two adapter plates and mounted on a Ro-tap sieve shaker. After a 30-minute sieving period, the dry sieve assembly is carefully dismantled. The screens are easily removed from the spacer ring by slipping the spacer rings over a rubber stopper in a shallow pan and carefully transferring the screens to counting cups for radio-assay.

Results and Discussion

LABORATORY RECOVERY TESTS

Data in Table II illustrate the efficiency of separating particles of different size ranges from varied depths of the granular collector matrix by wet sieving in isopropyl alcohol. An acceptable recovery of the fine particles from the 1.5 kg sample (one-inch depth) was achieved with a 5-minute wet sieving operation. Glass beads of the size ranges indicated, which were used to simulate fallout particles, while developing this process, were obtained from Minnesota Mining and Manufacturing Company, St. Paul, Minnesota. Since most fallout particles have been observed to be of a siliceous nature,[1, 3, 6, 8] it was assumed that these glass beads would simulate the wet sieving characteristics of fallout particles.

TABLE II

Recovery of Glass Beads from Varied Depths of Granular Collector Matrix During Five Minutes of Wet Sieving in Isopropyl Alcohol

Particle size (microns)	Per cent recovery from varied matrix depth*			
	½-inch	¾-inch	1-inch	2-inch
470	100	99.4	98.0	94.2
150	98.7	100	93.7	87.9
44	100	98.0	95.8	90.0

* 100 mg of each size particle was added to the matrix.

TABLE III

Recovery of Varied Amounts of Pre-Sized Glass Beads from a 30-Minute Sieving Period in the Dry Sieve Assembly

Sieve opening (micron)	Per cent recovery at various weight loadings per screen*					
	100-mg	250-mg	500-mg	1-gm	2-gm	3-gm
350	101.0	100.3	99.0	99.2	99.5	99.5
250	100.8	98.5	96.8	97.0	97.6	97.6
177	100.0	100.8	100.4	98.4	100.8	100.6
149	97.1	97.4	90.5	100.8	99.4	97.2
88	95.4	91.0	87.9	92.1	99.3	98.5
44	81.7	83.7	99.2	95.7	88.0	97.1
L.S.D. (P = .05)	17.8	17.4	14.0	9.5	4.6	6.32

* Mean of three replicates. Equal amounts of pre-sized beads for the six size ranges were added to the assembly at each weight loading. The per cent loss was recovered in the size range less than 44 microns in diameter.

In order to test the efficiency of recovering particulate material on the removable screens in the dry sieve assembly, equal amounts of various size ranges of pre-sized glass beads were mixed together at different weight loadings and sieved as described in the operational procedure. Recovery was determined gravimetrically (Table III). All of the different size ranges of particles normally fractionated from fallout contaminated soil samples were not available in glass beads; nevertheless, previous studies[1, 3, 6, 8] have shown that the particle size ranges tested are indicative of those in which fallout particles predominantly occur. Dry sieving time periods of 10, 20, and 30 minutes did not significantly affect the efficiency of recovering pre-sized particles at screen loadings less than 500 mg per screen; however, greater loadings of 1, 2 and 3 grams per screen required 30-minute sieving times for the most efficient recovery of particles. For this reason, a 30-minute sieving time was adopted as the standard to accomodate the amount of particulate materials normally expected to be collected from

the granular collector matrix of the surface area described.

The variations in recovery among the different screen sizes are attributable largely to the difficulty of accurately pre-sizing the glass beads, which are not perfectly round. This was particularly evident for particles less than 150 microns in diameter. Also, some fracturing of the particles into smaller portions was observed. The error variance was greater than the treatment or weight-loading variance; therefore, the effect of increasing weight loadings up to three grams per screen was not significant.

In previous studies[1, 8] the detailed delineation of fallout contamination and the particle size distribution in any given area of a fallout pattern was assessed from surface soil samples collected from the contaminated area. The standard procedure involved the fractionation of 100-gm lots of contaminated soil into 13 size ranges using 8-inch diameter standard testing sieves on Ro-tap shakers. It would be desirable to continue this means of delineating fallout patterns because of the logistically impractical problems of prelocating large numbers of fallout collectors in an anticipated fallout pattern. In order that particle size data from the soil surface samples and the granular collector samples might be compared, the particle size recovery characteristics of sieves 1½ inches and 8 inches in diameter were tested. The spacing between screens in a nest of 8-inch diameter sieves was one inch; the spacing between the 1½-inch diameter screens was ½ inch. Six different size ranges of pre-sized glass beads were mixed together and sieved for 30 minutes on the 1½-inch and 8-inch diameter standard testing sieves in amounts equivalent to a 500-mg weight loading per 1½-inch diameter screen area (Table IV). The difficulty of accurately pre-sizing particles that were not perfectly round and also the fracturing of particles into finer portions largely accounts for the variation observed. The results indicate that the 1½-inch and 8-inch diameter screens are equally efficient for dry-sieve particle sizing.

TABLE IV

Recovery of Pre-Sized Glass Beads on 1½-Inch and 8-Inch Diameter Screens from a 30-Minute Sieving Period

Sieve opening (micron)	Per cent recovery*	
	1½-inch screen	8-inch screen
350	99.0	94.6
250	96.8	99.0
177	100.4	99.9
149	90.5	97.3
88	87.9	91.3
44	99.2	95.4
L.S.D. (P – 0.05)	14.0	5.6

* Mean of three replicates. The per cent loss was recovered in the size range less than 44 microns in diameter.

TABLE V

Recovery of Fallout Debris from Granular Collector Matrix as a Function of Washings

Wash No.	Total particulate activity		0–44 μ Particle size activity	
	μc/sq ft (H + 12 Hrs)	per cent of total	μc/sq ft (H + 12 Hrs)	per cent of total
Replicate No. 1, Sample P-0709				
1	31.20	88.6	22.50	85.2
2	2.87	8.1	2.79	10.5
3	1.15	3.3	1.13	4.3
Replicate No. 2, Sample P-0709				
1	25.00	89.2	19.60	86.7
2	1.84	6.6	1.79	7.9
3	1.20	4.2	1.20	5.4

FIELD RECOVERY TESTS

The recovery of primary fallout debris from the granular collector matrix as a function of the number of washings in isopropyl alcohol is shown in Table V. Two washings were required to remove at least 95 per cent of the fallout activity under field processing conditions using the operational procedure described. Additional washings were not considered to be practical, although it was demonstrated that virtually all of the fallout activity could be removed upon repeated washings.

In addition to the granular type collector, gummed paper and resin-coated plate collectors also were tested for comparative measurements of fallout debris deposited in the natural environment. The gummed paper was Avery Adhesive Label Corp. No. 3. Metal plates were coated with an adhesive solution composed of 66.7 volume per cent toluene, 1.3 volume per cent tributyl phosphate and 32.0 volume per cent Du Pont RL-233 resin. Evaporation of toluene left an adhesive residue that remained tacky throughout the collection periods. Data in Table VI show comparisons of the relative capacities of the granular, resin plate and gummed paper collectors to measure the fallout debris deposited at different sampling stations in a typical fallout pattern. These data are representative of those obtained from nuclear detonations during the 1957 Test Series. For purposes of measuring

TABLE VI

Comparison of Fallout Contamination Measured by Different Types of Fallout Collectors Pre-Located in a Fallout Pattern Typical of the 1957 Weapons Testing Series

Station	Granular collector	Resin plate collector	Gummed paper collector	Soil surface
	$\mu c/ft^2$ at H + 12 Hrs			
0103	4.18	0.07	0.14	27.21
0108	852.00	98.20	394.00	499.00
0113	895.00	327.10	1,121.00	694.70
0118	56.90	23.81	75.10	15.35
0308	1,120.00	380.80	684.90	434.00
0311	215.00	164.90	241.10	—
0313	54.30	37.90	48.90	93.59
0318	4.60	0.02	0.04	26.14
0503	572.00	228.00	506.40	237.00
0508	56.60	48.10	82.20	49.20
0513	8.92	6.75	10.00	2.80
0518	3.70	2.93	5.00	6.35

the unit area activity deposited in a fallout pattern, reasonably good correlation occurred between the different kinds of collectors studied. The granular collector gave measurements comparable to the gummed paper measurements but these were often higher than those from the resin plate or soil surface. The main cause for deviation of collector measurements from soil samples is attributable to the difficulty of obtaining representative samples of fallout debris deposited on the soil surface. The tremendous dilution of fallout particles in the soil and the ensuing problems of self-absorption upon radio-assay are factors which contribute to the deviations observed upon comparing collector and soil surface measurements.

The modal distribution of the ratio of gummed paper to granular collector fallout debris measurements from 65 sampling stations in several fallout patterns is illustrated in Figure 5. About 58 per cent of the gummed paper collectors had higher unit area activity measurements than were found on the granular collectors. The influence of fallout particle size on the activity measurements observed between these two types of collectors was not detectable.

The granular collector provided an excellent means of studying the fallout particle distribution from different types of detonations. It also enabled some studies to be made on the efficiency of using standard testing sieves for determining fallout particle size distribution in samples of surface soil from fallout areas. It was observed that greater amounts of fallout particles of size range less than 100 microns in diameter were present in debris from the 500-ft balloon detonations than from the 500-ft tower detonations (Figure 6). Also, it was apparent that a large portion of the very fine fallout particles that had been deposited on the soil surface was retained in the larger particle size range fractions during the dry sieving of soil samples probably, by an adherence of the fine particles on larger, inert soil particles. The dry sieved surface soil samples collected from fallout areas were not as reliable as the granular collector for measuring particle size distribution when the major portion of the fallout debris was of size range less than 150 microns in diameter. Results obtained with the granular collector suggest that greater

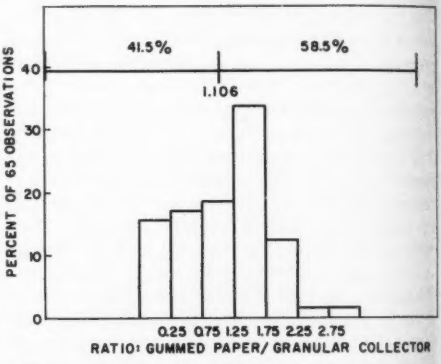

FIGURE 5. Modal distribution of the ratio of gummed paper to granular collector fallout debris measurements at 65 sampling stations.

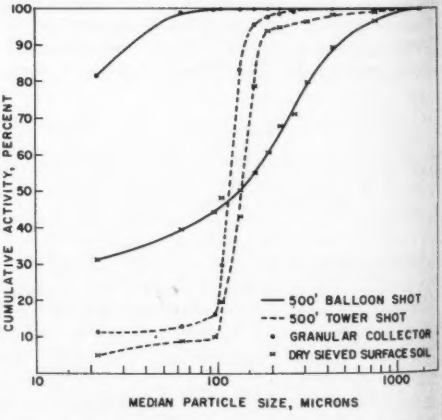

FIGURE 6. Comparison of particle size distribution in fallout debris from 500 ft balloon and tower detonations as measured by the granular collector and the dry sieve analysis of surface soils.

amounts of fallout particles of size range less than 150 microns in diameter might have been deposited from nuclear detonations during previous weapons testing series than was indicated by dry sieved surface soil samples collected from fallout areas. These very fine fallout particles are of greater biological significance than large particles.[6]

Summary

A granular collector for sampling fallout debris from nuclear detonations was developed which consists of a shallow, metal tray and a removable mylar plastic liner filled with ⅛-inch diameter polyethylene plastic pellets. Fallout debris impinging upon the exposed collector tray during fallout is physically entrapped within the granular matrix and later separated from the matrix by wet sieving in isopropyl alcohol. The fallout debris may be fractionated into different particle size ranges by dry sieving with standard testing sieves. An operational procedure for recovering the primary fallout debris from the granular matrix is described.

The efficiency of the recovery procedure was tested using pre-sized glass beads, soil samples and fallout debris. Virtually all particulate material smaller than the granular matrix could be removed by wet sieving in isopropyl alcohol. Less than two per cent of the sample was lost during a dry sieving process of fractionating the particulate material into 13 different particle size ranges. A recovery of more than 95 per cent of the samples processed through the operational procedure was consistently obtained.

The granular fallout collector was used extensively during the 1957 Weapons Testing Series in areas adjacent to the Nevada Test Site in conjunction with Program 37, CETG. It compared favorably with the gummed paper and was superior to resin-coated tray collectors and soil surface samples as a means of collecting fallout; it was superior to all of these collectors with respect to the ease of separation of primary fallout particles for physical and chemical characterization and for bioassays. The granular collector is economical for widespread coverage of anticipated fallout areas.

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Rutgers Air Pollution Courses

DURING THE 1959–1960 ACADEMIC YEAR two courses in Air Pollution will be offered by Rutgers University, New Brunswick, New Jersey. In the first term a three credit course, *Principles of Air Pollution Control,* is designed to familiarize the student with fundamental factors responsible for atmospheric contamination, the effects of pollution on man and his environment, basic principles of measurement, methods of control, and some knowledge of the legal aspects.

The second term course, *Air Sampling and Analysis,* will be a two-credit laboratory course providing training in the basic techniques of sampling and analysis of community atmospheres, source emissions and vegetation. Meteorologic methods and actual field surveys will be included.

Registration for these courses should be made before September 16, 1959 for the first term and January 29, 1960 for the second course. For detailed information address The Department of Sanitation, Rutgers University, New Brunswick, New Jersey.

Experimental Cancers in Rats Produced by Chromium Compounds and Their Significance to Industry and Public Health*

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THE DESIGN and application of suitable and effective technologic and sanitary measures for protection of the producers and consumers of carcinogenic chemicals, especially when present in mixtures or formed during some undetermined phase of a manufacturing process is greatly facilitated by the availability of adequate information concerning the exact nature of such chemicals. Data concerning their identity are, therefore, of distinct industrial importance. The existence of a serious lung cancer hazard for producers of chromates from chromite ore and for handlers of certain chromium pigments (zinc chromate, barium chromate and lead chromate) has been known for about 25 years from observations made among workers employed in German, American and English plants.[1, 3, 4, 6, 9, 10, 11, 14]

Despite the existence of adequate epidemiologic evidence as to the reality and extent of this occupational cancer hazard in these operations, it has remained uncertain whether these cancers are caused by trivalent or hexavalent chromium compounds, by water soluble or insoluble derivatives, by monochromates or dichromates, by chromite ore, intermediary roasting products or chromium metal, or by chromium in all these forms under proper conditions of exposure. There has remained even the possibility that these tumors were causally not related to chromium at all, but to contact with other constituents of the chromite ore, such as iron and vanadium, or to exposure to incomplete combustion products of the fuel (bunker C fuel oil) extensively used in the roasting process of the ore.

Past attempts to produce cancers in experimental animals (mice, rats, guinea pigs and rabbits) by inhalation or parenteral introduction of metallic chromium, chromite ore, and various chromium compounds gave negative or equivocal results.[2, 5, 8, 13] However, Hueper[8] recently reported the development of squamous cell carcinomas of the lung and sarcomas of the lung and of the soft tissues of the thigh of rats which had received intrapleural or intramuscular implants of powdered chromite ore roast suspended in sheep fat. Although the results of these experiments merely suggested that chromium might be the specific causal agent active in the production of these tumors, this interpretation received support from subsequent investigations briefly referred to in which it was shown that similar neoplastic responses followed a similar parenteral introduction of highly pure calcium chromate and of sintered calcium chromate.

It was concluded from this evidence that chromium is a carcinogenic agent when it is present in tissues in a form which provides for its adequate biological availability. This biologic availability appears to depend on the degree of solubility of the chromium compound in the particular biologic medium, i.e., the proper rate of release of chromium ion from the introduced chromium compound as well as the absolute amount of chromium compound present and its distribution in the tissues. A carcinogenic action of chromium compounds seems to depend upon an amount of chromium biologically available which is adequate to elicit a specific biologic reaction but insufficient to produce and continue cell necrosis.

For testing the validity of this thesis a series of experiments was undertaken. The results of the first set of experiments are presented in this communication.

Experimental Procedures

The following compounds were implanted intramuscularly and intrapleurally in Bethesda Black strain rats: calcium chromate, sintered calcium chromate, sintered chromium trioxide (chromic chromate), and barium chromate. Each compound was finely pulverized and contained less than 1 per cent of impurities. The sintered calcium chromate and sintered chromium trioxide were prepared by heating the respective compounds to about 2000°F. for one hour.

* Presented at the Twentieth Annual Meeting of the American Industrial Hygiene Association, April 25-May 1, 1959, Chicago, Illinois.

TABLE I

Death Distribution and Tumors Observed in Rats at Site of Implants of Chromium Compounds and of Sheep Fat Only

Compound	Site of implant	Deaths in periods of months after administration of material								Duration of exposure months	No. dead	No tumor at site	% of dead with tumors
		0–3		4–6		7–9		10–12					
		with tumor	no tumor	with tumor	no tumor	with tumor	no tumor	with tumor	no tumor				
$CaCrO_4$	Thigh	0	1	3	4	5	0	0	0	13	13	8	62
	Pleural	0	3	7	1	13	5	1	5	12	35	21	60
Sintered	Thigh	0	1	6	2	2	1	0	2	14	14	8	57
$CaCrO_4$	Pleural	0	2	3	4	13	2	1	1	14	26	17	65
Sintered	Thigh	0	0	4	3	8	0	3	1	12	19	15	79
CrO_3	Pleural	0	1	2	1	8	2	4	2	12	20	14	70
$BaCrO_4$	Thigh	0	2	0	1	0	1	0	1	12	5	0	0
	Pleural	0	0	0	3	0	1	0	1	12	5	0	0
Sheep	Thigh	0	1	0	1	0	1	0	3	12	6	0	0
Fat	Pleural	0	0	0	1	0	3	0	1	12	5	0	0

No. of rats at beginning of experiment:—35 (20 male and 15 female) for each material at each site.

The sintering process results in the reduction of part of the hexavalent chromium to lower valences, such as chromic chromate ($xCr_2O_3 \cdot yCrO_3 \cdot zH_2O$ or Cr_5O_{12}). In both sintered CrO_3 and sintered $CaCrO_4$ some of the original material remains unreacted.

Each material was mixed with sheep fat obtained by extracting fresh lamb fat with ether in a Soxhlet apparatus and evaporating the ether under vacuum. The materials were mixed in the proportion of 25 mg of the chromium compound to 50 mg of fat and formed into small cylindrical pellets weighing 75 mg. For each compound, pellets were implanted through an incision in the intercostal tissue of the right lateral aspect into the pleural cavities of 20 male and 15 female rats about three months old. The wound was closed by cotton sutures of the muscle layer and of the skin. Similar groups of the same age and strain received identical implants into the muscle tissue of their right thigh. Two groups of 20 male and 15 female rats were implanted with pellets of sheep fat only into the pleural cavity and into the thigh, respectively. Animals were killed when firm masses were observed in the region of the right thigh or, in the case of those receiving intrapleural implants, when palpation indicated the presence of a firm mass in the pleural cavities or, in some instances, when x-ray films of the chest of these rats made on several occasions revealed the presence of opacities in the right pleural space, suggesting a neoplastic growth.

The results obtained in these experiments during an observation period ranging from 12 to 14 months for the different groups are summarized in Table I according to relative length of exposure time and tumor yield. The data presented show that calcium chromate, sintered calcium chromate and chromic chromate produced within the stated observation period tumors at the site of implantation in 60 to 79 per cent of the rats which have died so far.

The first tumors involving either the thigh or the pleural cavity were observed toward the end of the fourth month following the implantation, when the rats were about seven months old. These tumors measured, at that time, from one to three centimeters in diameter, were firm in consistency, and in part relatively well delineated from the surrounding tissue except at their base where they were fixed to the periosteal tissue of the femur or the tissues of the mediastinum. When later in the experiment more advanced tumors were seen, invasion of the bony pelvic girdle and the lumbar spine and of the thoracic wall, the ribs, the lungs and heart, the diaphragm and the liver were observed together with metastatic nodes in the mediastinal and, occasionally, axillary lymph nodes. The tumors ranged in size from one to four or five cm in diameter and had generally a rather homogeneous, white cut surface. Since the intrapleurally implanted material seemed to settle preferably in the anterior lower angle of the right pleural cavity, this location appeared to be the most frequent site of the neoplasms obtained. In most instances, either extensive yellow or black colored spots representing residual material of the various chromium compounds used, were present within the neoplastic parenchyma (Figure 1).

The histologic examination of the tumors revealed that with the exception of one neoplasm involving the lung which was a squamous cell carcinoma (Figure 2), all others were sarcomas,

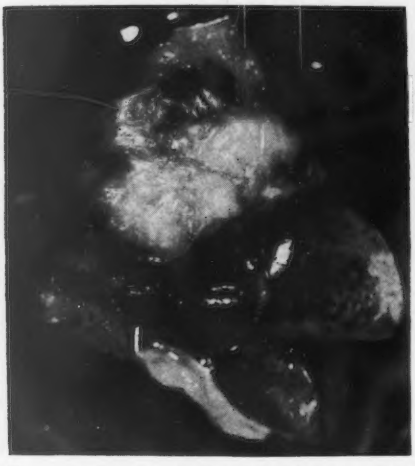

FIGURE 1. Sarcoma in the right pleural cavity of a rat following an intrapleural implant of calcium chromate retained in part in the center of the tumor as a yellow pellet.

FIGURE 2. Squamous cell carcinoma in the lung of a rat following implantation of a pellet of calcium chromate. Mag. 235×

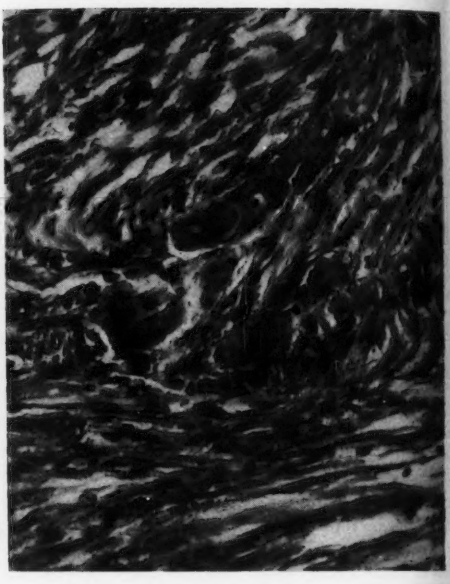

FIGURE 3. Spindle cell sarcoma of the thigh with myoblastic clusters after intramuscular implantation of calcium chromate. Mag. 251×

usually of the spindle cell- or fibro-sarcomatous types (Figure 3). Some of them were highly anaplastic and contained numerous grotesque giant cells (Figure 4). Sarcomas produced by sintered calcium chromate contained within the tumor tissue amorphous black pigment either in large clusters or in the form of a fine dust (Figure 5). In tumors elicited by chromic chromate the implanted material was demonstrable in the parenchyma in the form of black crystals (Figure 6). Some of the sarcomas invaded by contiguous growth the lung (Figure 7) and the heart (Figure 8).

Comments

The location of the tumors obtained with calcium chromate, sintered calcium chromate and chromic chromate, their relation to the implants, and the surprisingly early and high yield, leave no doubt as to the existence of causal relations between the implanted chromium compounds and the subsequently appearing cancers. These results, moreover, prove that the carcinogenic agent contained in the materials tested is represented by chromium, since chromium is the element common to the various compounds eliciting cancers. The carcinogenic effects produced

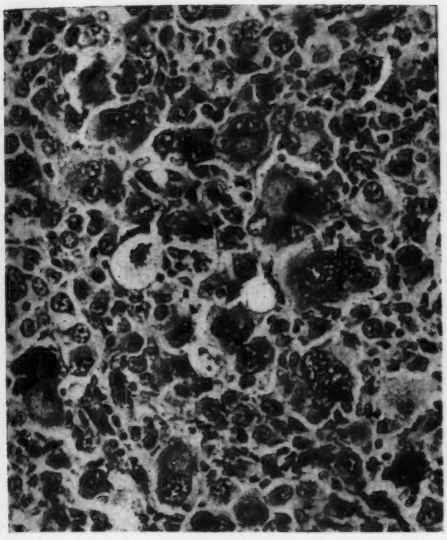

FIGURE 4. Anaplastic giant cell sarcoma in the thigh muscle of a rat after implantation of chromic chromate. Mag. 246×

FIGURE 6. Spindle cell sarcoma in the thigh with scattered grey-black crystals of sintered calcium chromate. Mag. 240×

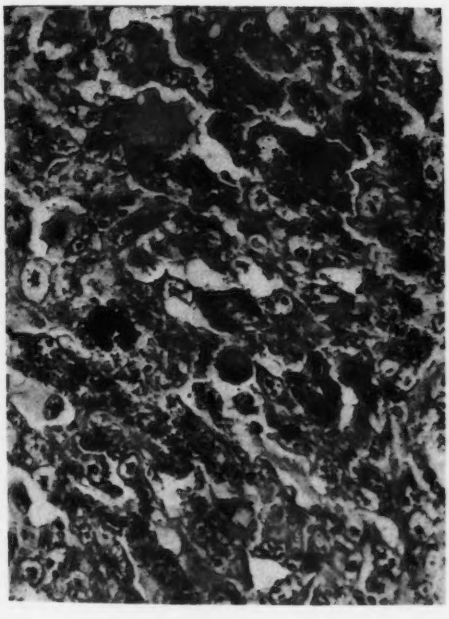

FIGURE 5. Anaplastic sarcoma in the thigh muscle with extensive black deposits of implanted chromic chromate. Mag. 245×

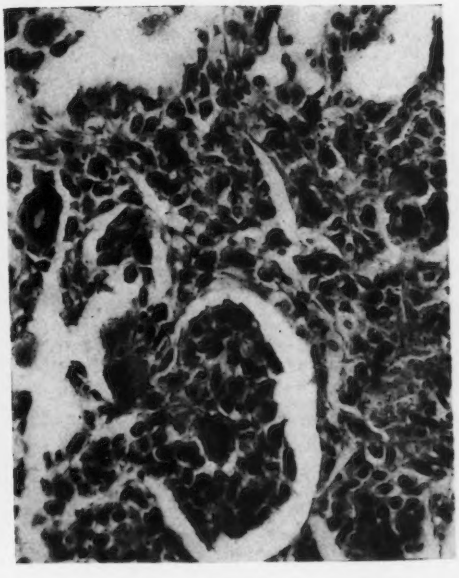

FIGURE 7. Anaplastic sarcoma of the right pleural cavity invading the lung, and produced by sintered calcium chromate. Mag. 245×

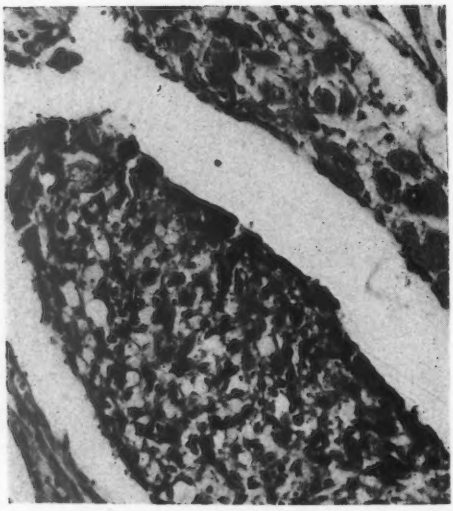

FIGURE 8. Irregular cell sarcoma invading the myocardium including the papillary muscle and extending from a sarcoma located in the right pleural cavity which was produced by implanted sintered calcium chromate. Mag. 215×

TABLE II

Solubility of Chromium Compounds in Water and Ringer's Solution

Compound	Solubility in micrograms of hexavalent chromium per ml of water or Ringer's solution at 37°C.		Carcinogenicity to rats
	Water	Ringer's	% of dead with tumors
Calcium chromate $CaCrO_4$	2470	3040	61
Sintered calcium chromate	1280	1500	61
Sintered CrO_3 (Cr_3O_{12})	1710	1780	75
Zinc chromate $ZnCrO_4$	610	830	?
Strotium chromate $SrCrO_4$	230	320	(40)*
Barium chromate $BaCrO_4$	8.5	9.0	0
Lead chromate $PbCrO_4$	<1	<1	?

* Preliminary results based on small number of early deaths.

by sintered chromium trioxide are, in this respect, especially significant.

The observations made indicate, moreover, that sheep fat, the vehicle used, has under the experimental conditions applied, no carcinogenic properties. The negative results obtained so far with barium chromate point to the importance of the relative degree of water solubility of chromium compounds in determining their carcinogenic effects. As shown in Table II, barium chromate is considerably less soluble than the chromates which caused the development of sarcomas and carcinomas. The validity of this general principle determining the biologic availability of chromium becomes even more definite in the light of the carcinogenic effects elicited by strontium chromate using the above experimental approach, which is now being applied to additional hexavalent chromium compounds, some of which are listed in Table II, and to some trivalent compounds. These current studies are performed also for ascertaining whether or not in addition to biologic availability, the valence of the chromium ion is of any importance in controlling the occurrence and degree of a carcinogenic effect.

This aspect of the problem of chromium cancer is not only of distinct scientific importance but even more so from an industrial viewpoint.[7, 8, 10] The valence of the chromium determines to some extent its diffusibility through cell membranes hexavalent chromium being readily diffusible through the erythrocytic cell membrane, while trivalent is largely nondiffusible. Trivalent chromium in turn, is strongly bound to proteins, while hexavalent chromium is only slightly and unstably bound to proteins at physiologic pH. From a carcinogenic viewpoint, it may be significant that trivalent chromium seems first to be important by providing the extracellular initial depot from which continuously, through metabolic processes, small or even minute amounts of solubilized chromium in hexavalent form may be released into the cells where this then is converted back into biologically active, protein bound, stable trivalent chromium. This concept of chromium carcinogenesis was first proposed by Mancuso and Hueper[10] (1951) when discussing the apparent and possible role which the inhalation of dust of chromite ore might play in the production of lung cancer among chromate workers. Evidence supporting this concept was subsequently obtained by Hueper[8] (1952) on rats which after exposure for 18 months to the inhalation of finely powdered chromite ore, exhibited an excessive chromium level of the blood (13 to 16 gamma). This finding proved that a fraction of the chromium in the ore introduced into the bronchial tree had been solubilized and had entered the blood by penetrating the bronchial tissues. Further confirmation and elabora-

tion of this fundamental concept was subsequently provided by the investigations of Grogan[7] (1957) which dealt with the relative solubility of chromium in chromite ore and metallic chromium in water, Ringer's solution, Locke's solution and phosphate buffer with added bicarbonate under conditions of oxygenation, aeration and nitrogenation and with quantitative studies on the chromium contents of blood and tissues of rabbits and dogs after repeated intratracheal insufflation of these chemicals.

The present experiments demonstrate that several chromates with medium solubility, when introduced into the tissues of rats in the form of a depot assuring thereby conditions of prolonged exposure to chromium in rather small amounts produce cancers. The experimental conditions, however, do not give any information on the industrially important problem as to whether or not an often repeated introduction of very small and presumably noncorrosive amounts of highly soluble chromates, such as sodium chromate, under otherwise identical experimental conditions, might in the course of many months also elicit a neoplastic response from the tissues exposed to chromium compounds especially known for their corrosive necrotizing properties. Because of its industrial significance this aspect is being investigated in experiments at present being conducted. Preliminary observations already on hand in regard to the carcinogenic action of strontium chromate make it likely that the slightly more soluble zinc chromate is also carcinogenic.

The relatively low yield on carcinomas of the lung resulting from an intrapleural introduction of the carcinogenic chromates made it necessary to reinvestigate this aspect by using a technique recently applied by Della Porta et al.[12] for the production of pulmonary carcinomas in hamsters. Very finely powdered calcium chromate suspended in a gelatine solution is being instilled for this reason, into the bronchial lumen of rats through a catheter in bi-monthly intervals. The results of these and related experiments on chromium carcinogenesis will be reported at a later date.

Conclusions

1. Calcium chromate, sintered calcium chromate and sintered chromium trioxide when introduced in pellet form into the muscle tissue of the thigh and into the pleural cavity of rats produce cancers, mainly sarcomas, in the majority of animals at the site of implantation within 14 months. They made their first appearance as early as five months after implantation.

2. This effect is related to the degree of solubility of these compounds in a watery medium since rats treated under identical conditions with barium chromate, which has a low degree of solubility, did not develop during the same period any tumors.

3. These observations lend support to the concept, that the degree of biologic availability as determined by the solubility of a chromium compound and the amount present controls largely the carcinogenicity of these compounds under the experimental conditions used.

4. The results reported establish the fact that chromium as such is a carcinogenic agent since it is capable of producing carcinomas of the lung and sarcomas of the soft tissues of the mediastinum and thigh.

5. The observations reported make it likely that zinc chromate which is widely used as an anticorrosive paint pigment, also possesses carcinogenic properties because it is slightly more soluble than the carcinogenic strontium chromate.

6. The application of these data will help industry to assess more intelligently potential carcinogenic properties of chromium compounds and to apply proper protective and preventive technologic and sanitary measures.

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PERMISSIBLE LIMITS FOR RADIONUCLIDES

THE NATIONAL BUREAU OF STANDARDS Handbook 69, *Maximum Permissible Body Burdens and Maximum Permissible Concentrations of Radionuclides in Air and Water for Occupational Exposures* was issued June 5, 1959 and supersedes NBS Handbook 52.

This new Handbook contains the latest recommendations of the National Committee on Radiation Protection and Measurements (NCRP) concerning the maximum permissible internal exposure of the human body to radioisotopes. These recommendations represent a revision of those published by the NCRP in 1953, which were for occupational exposure, 24 hours per day, continuously for 70 years. Prepared by the Subcommittee on Permissible Internal Dose, the study took five years to complete and included nearly 75 radioisotopes. The work has continued at an accelerated pace since 1953 with the added cooperation of the International Commission on Radiological Protection (ICRP). The present Handbook includes maximum permissible concentrations (MPC) and body burdens for some 240 radioisotopes and includes many refinements based on knowledge not available in 1953. The study has included the evaluation of nearly 2000 separate researches reported in the literature, having bearing on the permissible dose problem.

The new values reflect, where applicable, the lowering of the basic maximum permissible dose (MPD) recommended by the NCRP in 1957 and 1958. As a result of all of these considerations, some values have been increased, some decreased, and others remain essentially the same. In addition, the calculations have been changed from 70 years continuous occupational exposure to 50 years continuous occupational exposure, a more realistic figure for radiation workers. All maximum permissible concentrations (MPC) are given for both 40- and 168-hour weeks.

The recommendations in Handbook 69 are to be considered only as recommendations of a group of experts in the radiological protection field. They carry no legal force requiring or demanding adoption. This publication may be purchased from the Superintendent of Documents, U. S. Government Printing Office, Washington 25, D.C., for 35 cents.

PROCEEDINGS OF TRITIUM SYMPOSIUM

THE SYMPOSIUM on Advances in Tracer Applications of Tritium was held in New York City on October 31, 1958. As a sequel to a similar symposium held in 1957, it was intended to present the latest information available in terms of specific applications. The sponsors, New England Nuclear Corporation, Packard Instrument Co., Inc., and Atomic Associates have published a condensed form of the proceedings. This 67 page booklet contains summaries of eleven papers covering various reactions, uses, and methods of measurement related to tritium. The booklet and further information may be obtained free on request from New England Nuclear Corporation, 575 Albany Street, Boston 18, Massachusetts.

The Determination of Zirconium in Air*

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Health Division, Los Alamos Scientific Laboratory, University of California, Los Alamos, New Mexico

THE PURPOSE of this study was to apply the Bricker and Waterbury method[1] to the analysis of air samples of zirconium, which in industrial plants is predominantly in an insoluble form from machining or powder metallurgical operations. The Hygienic Guide for zirconium[2] suggests this method as a means of evaluating possible zirconium exposure. The threshold limit of 5 mg/M^3 makes an ultramicro method unnecessary; however, because atmospheric contamination during some operations is of short duration, levels below the milligram level are of interest. The range of 1 to 10 μg of zirconium per sample was chosen for this study, which for a 10-liter air sample means that from 0.1 to 1 mg of zirconium per cubic meter may be determined.

Reagents

All of the chemicals used were commercially available reagent grade unless otherwise specified.

1:1:3 Digestion mixture. To 300 ml of concentrated nitric acid add 100 ml of concentrated sulfuric acid; cool to room temperature and add 100 ml of perchloric acid (71 per cent, sp.gr. 1.768).

1:5 Digestion mixture. To 100 ml of concentrated nitric acid add 20 ml of 71 per cent perchloric acid.

Hydrochloric acid 3.5M. To 500 ml of distilled water add 286 ml of concentrated hydrochloric acid and dilute to one liter with distilled water.

Saturated hydroxylamine hydrochloride. Dissolve approximately 100 gm of hydroxylamine hydrochloride in 100 ml of distilled water; store in a brown bottle in a cool place.

1:1 Sulfuric acid. To 50 ml of distilled water add slowly 50 ml of concentrated sulfuric acid (H_2SO_4, 96 per cent, sp.gr. 1.84); cool; store in a dropping bottle.

p-Bromomandelic acid, 2 per cent. Dissolve 2 gm of the reagent ($C_6H_4Br—CHOH—CO_2H$, Pilot Chemicals, Inc.) in 100 ml of warm distilled water. Cool the mixture and filter into a storage bottle.

Perchloric acid, 1.5M. To several hundred milliliters of distilled water add 120 ml of perchloric acid (71 per cent $HClO_4$, sp.gr. 1.768) and dilute to one liter. (*NOTE:* The precise normality is not so essential as the use of the same normality throughout each set of determinations.)

Saturated chloranilic acid. (2,5-dichloro-3,6-dihydroxy-*p*-quinone, Eastman Organic Chemicals #P-4539). Purify the reagent by dissolving 8 gm of the practical grade chemical in a liter of boiling water; filter the solution while hot. Cool the filtrate to 50°C and extract with two 200-ml portions of benzene. Carry out the extractions only in a well-ventilated hood. Discard the benzene extracts; cool the aqueous phase in an ice bath or allow to stand overnight. Filter the solution and wash the crystals with not more than three 10-ml portions of distilled water. Dry the crystals at 115°C. To prepare the working solution of chloranilic acid, add 200 ml of 1.5*M* perchloric acid to 50 mg of the purified reagent. The reagent is stable for several weeks after mixing. Before use, decant or filter a sufficient volume for all standards and unknowns.

Stock zirconium standard. There are other methods of preparing a more precise stock zirconium standard,[3] but zirconyl nitrate is simple to use and may be standardized for greater accuracy. Dissolve 0.733 gm of zirconyl nitrate ($ZrO(NO_3)_2 \cdot 2H_2O$, AR) in 1.5*M* perchloric acid and dilute to one liter; 1 ml = 250 μg of zirconium.

Working zirconium standard. Dilute 10 ml of the stock zirconium standard to 500 ml with 1.5*M* perchloric acid; 1 ml = 5 μg of zirconium.

Preparation of Standard Curve

To six 10-ml volumetric flasks add 0, 0.5, 1, 2, 4, and 5 ml of the working standard; to each flask add one ml of the decanted chloranilic acid reagent and dilute to volume with 1.5*M* perchloric acid. Mix each flask, and after 15 minutes read the optical density of the solution in a one-centimeter Beckman cuvette at 340 mμ on a Beckman DU spectrophotometer. Use 1.5*M* perchloric acid as the reference solution. The flasks contain 0, 2.5, 5, 10, 20, and 25 μg of zirconium, respectively. Figure 1, Point A, shows a plot of the concentration of zirconium vs optical density.

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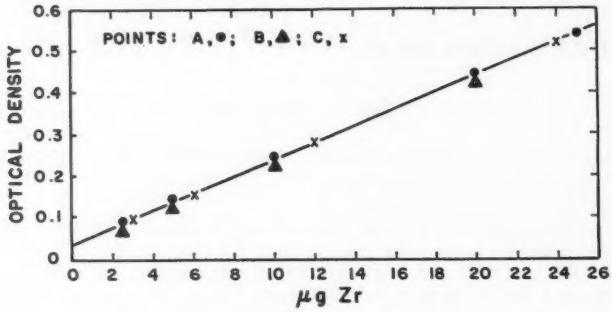

FIGURE 1. Standard curve.

TABLE I

Turbidity and Dilution Requirements

Turbidity 5 min. after heating	Zr μg	Solution vol. ml	Aliquot for color development
None	0 to 10	6 to 7	All
Visible	10 to 40	Make to 10	5 ml
Heavy	40 to 100	Make to 25	5 ml
Flocculation	over 100	Make to 25	1 to 5 ml

Analytical Procedure

Place each of the filter paper samples (1⅛ or 2⅛ inch Whatman #41) in a 40-ml short, conical, centrifuge tube. To five similar tubes add 0, 0.5, 1, 2, and 4 ml of the working standard; the tubes contain 0, 2.5, 5, 10, and 20 μg of zirconium, respectively. Evaporate the standards to dryness or to fumes of perchloric acid, and cool. To each standard add a filter paper the same size as that of the unknown sample. To all the tubes add three ml of the 1:1:3 digestion mixture. Place the tubes in a sand-filled aluminum block at 200°C until the filter paper dissolves; then transfer the tubes to a 350°C block and heat until the filter paper is ashed and only a small amount of sulfuric acid is condensed on the upper wall of the tubes. Cool the tubes and add one drop of 1:1 sulfuric acid in distilled water and several drops of concentrated hydrochloric acid. Evaporate each to definite fumes of sulfuric acid at 200°C, then cool the tubes. Carefully add three ml of 3.5*M* hydrochloric acid and warm to 100°C in an aluminum block; while warm add one ml of hydroxylamine hydrochloride and continue the heating for 30 minutes. Swirl the tubes occasionally. Add three ml of p-bromomandelic acid and continue heating for 30 minutes. After five minutes check the tubes for turbidity to determine the need for diluting the final solution (Table I). Cool the tubes to room temperature and wash down the walls of each with six to ten drops of Braunsol (Braun Corp.). Swirl the tube, then wash down the walls with not more than one ml of distilled water. Centrifuge the tubes, carefully remove the supernatant fluid by aspiration, and discard it. Again, wash down the walls of the tube with four drops of Braunsol and several milliliters of distilled water. Resuspend as much of the precipitate as possible and again centrifuge. Remove and discard the supernatant fluid as before. To each tube add six drops of 1:5 digestion mixture and heat in an aluminum block for 15 to 30 minutes or until fumes of perchloric acid are clearly visible and the residues are dry. (Do not heat the tubes for extended periods.) Cool the tubes, add three ml of 1.5*M* perchloric acid and warm to 100°C; carefully decant, or transfer to 10-ml volumetric flasks with a pipette. Rinse each tube with three to four one-ml portions of 1.5*M* perchloric acid. If the preliminary estimation (see Discussion and Table I) indicates more than 25 μg of zirconium, use an aliquot of this solution before adding the color reagent. To each flask add one ml of filtered chloranilic acid and bring to volume with 1.5*M* perchloric acid. Mix the contents of each flask well and determine the optical density of the solution at 340 mμ in a one-centimeter Beckman cuvette as described under preparation of the standard curve. Plot the optical density vs concentration for the standards carried through the

TABLE II

Recovery Range

Zr concentration μg	% recovered
5	100 ± 13
10	90 ± 10
20	95 ± 6
25 or more	98.4 ± 2.7*

* Bricker and Waterbury.

procedure, and from this curve (Figure 1, Points B) determine the concentration of the samples.

Discussion

The techniques are fully discussed in the original work by Bricker and Waterbury; however, the modifications made here should be explained in detail. A consistent strength of perchloric acid, and preferably from a single batch, is important. Unless the acid is standardized with meticulous care, there will be a noticeable difference in replicate analyses. The optical density of 340 mμ was chosen on the basis of a greater sensitivity, although it does not represent the maximum absorption of the colored complex. The blank becomes significantly high when the acid concentration is decreased or when the wave length is less than 340 mμ.

The precipitation of zirconium as the p-bromomandelic acid complex makes the method specific and also offers a means of estimating the amount of zirconium present so that a proper aliquot can be taken. Table I indicates the type of dilutions that can be used for samples with amounts in excess of the standard curve.

Sensitivity of the method depends upon two main factors. One is the solubility of the zirconium bromomandelate and the other is the dissolution of the zirconium after ashing the precipitated zirconium complex. As little as 5 μg of zirconium will be precipitated by the reagent without coprecipitants. Zirconium-95 was used to determine the efficiency of the precipitation. The solution of Zr^{95} was made so that each milliliter was equivalent to 5 μg of zirconium with 150,000 d/m/ml (disintegrations per minute per milliliter). The zirconium was virtually all precipitated. The use of celite or other carriers caused a loss in recovery because of the added number of washings and centrifugings needed to separate the zirconium. Adding concentrated hydrochloric acid to the ashed residue aids the dissolution of the zirconium.

The recovery of zirconium from spiked filter paper is dependent upon the concentration of the zirconium. The range of precision that may be expected is given in Table II.

The increased precision of the method, as indicated by the original workers,[1] was confirmed. A standard curve, prepared by using a standard 25 μg of zirconium per milliliter, is shown in Figure I, Points C. Three-milliliter aliquots were used and diluted to 10 ml for final color formation and reading.

The variation in the blanks or zero zirconium standards carried through the procedure is due to the slight shift of the acid concentration and the effect of light on the chloranilic acid solution. The optical density of the blank varied between 0.030 and 0.050 during a two-week period, but remained constant throughout any single analysis. Blanks varied from 0.031 to 0.036 in a series of four separate analyses if the chloranilic acid solution was decanted or filtered just before use.

A standard curve carried through the entire procedure corrects each value for recovery and eliminates correction factors.

Attempts were made to apply this technique of isolation and colorimetric measurement of zirconium to urine samples. The presence of phosphates, however, makes it necessary to isolate the zirconium before this analysis. A procedure using fluorides lacks the necessary sensitivity for such an analysis. There is no simple effective method of isolation at this time.

The modifications of the Bricker and Waterbury method, necessary for the determination of zirconium in air, are satisfactory and make the method simple enough for routine air analysis.

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The Toxicity of Boron Oxide*

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Introduction

ALTHOUGH MUCH research on the toxicology and pharmacology of high energy fuels[1-5] has been carried out in recent years, little attention has been paid to one of the main jet engine exhaust products, boron oxide. With current plans for increased use of these high energy fuels[6-8] it is not unlikely that a considerable amount of boron oxide, as the solid exhaust product, will be dispersed throughout the atmosphere and over wide areas adjacent to air fields and on flight decks.

Boron oxide, an anhydride, reacts exothermically with water to form boric acid. Although boric acid has been reported to be relatively nontoxic,[9, 10] there may be an exothermic reaction within the respiratory tract following inhalation of boron oxide particles. In this paper are reported results of studies of repeated inhalation, ad libitum drinking, and cutaneous, intragastric, and ocular administration of boron oxide.

Methods

Groups of albino rats and dogs were exposed to aerosols of boron oxide in four dynamic chambers having volumes of 20, 100, 1000 and 1000 liters. The rats were individually caged in racks, ten cages each, which were randomly changed for each exposure. The animals were exposed for six hours per day for five days per week. Boron oxide, which was pre-sized, was dispersed from modified Wright Dust[11] dispersers into the chambers at a fairly constant rate throughout the exposure period. Large particles were eliminated by means of a settling column between the disperser and the mixing bowl, where air entered the top of the chamber. A flow of room air was maintained through the chambers of about one-half of the chamber volume per minute.

Samples for determination of airborne concentrations of boron oxide were withdrawn from the chambers every hour and collected by means of a filter paper sampler containing 5/8-inch discs of Knowlton filter paper, Grade 100. The boron oxide was dissolved in water and estimated by the carmine-sulfuric acid method.[12] Standard solutions of boron oxide were run with every set to reduce possible errors from variations in the time of color development, acid concentration, or temperature. Samples for particle size determinations of the aerosol were collected by means of a modified Cascade impactor,[13] and mass-median diameters (MMD) were derived by use of predetermined stage calibrations for boron oxide.[14]

At two-week intervals, groups of exposed and control rats were placed in metabolism cages for urine collection, then sacrificed for samples of blood, tissue, and bone. Tissue samples were frozen for subsequent boron analyses and preserved for histopathological examination. The bones were stored in a desiccator for fragility tests.

Urinalysis included volume, pH, sugar, albumin, creatinine coefficient, and boron content.

The fragility of the dried femurs of rats was measured by use of a "Modulimeter." The individual bone was supported at each end while a dull blade rested on its center. Increasing weight was added to the pan attached to the blade until the bone broke into two or more pieces. The ratio of the fracture weight (kg) to the least diameter (mm) was considered an index of bone fragility.

Roentgenograms of control and exposed rats were taken for comparison of bone structure. Chemical analyses of blood for sugar, protein, creatine, lactic acid, inorganic phosphorous, and cholesterol were performed. Hematologic examinations consisted of total red and white blood cell counts, hemoglobin, hematocrit, and differential counts.

The dogs were bled every two weeks for chemical and hematological analysis. On alternate weeks they were tested for liver function by the standard method of sulfobromophthalein clearance. Body weight determinations of individual

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animals exposed to B_2O_3 aerosols, and their controls, were made weekly.

Four groups of ten rats each were fed, ad libitum, water containing boron oxide in concentrations of 0.25, 0.50, 1.0, and 1.5 per cent by weight. Their daily water consumption and body weights were recorded as well as similar data for a control group. In addition, another group of ten rats received a ten per cent slurry of boron oxide in water daily by intragastric intubation for a period of three weeks, five days per week.

In order to determine any cutaneous effect, boron oxide dust (1 gm/25 cm^2) was applied to wetted backs of four rabbits that had been clipped the previous day.

About 50 mg of the oxide was placed in the left eye of four rabbits to determine ocular effects.

Results and Discussion

The physical exposure data, including chamber size, concentration, particle size, and exposure times, are presented in Table I. The cumulative weight curve of the dust dispersions is shown in Figure 1.

At no time were any toxic signs noticed, nor were there any deaths due to inhalation of the boron oxide aerosol. However, some of the rats exposed to a concentration of 470 mg/m^3 had a slight reddish exudate from the nose. Since these animals were covered with the dust, there was probably local irritation of the external nares and scratching. This concentration produced a dense cloud of fine particles. Workers experienced in the aerosol field expressed their belief that visibility in such a cloud would probably be limited to ten or twelve feet.

The weight changes of control and exposed animals are shown in Figure 2. Since female rats had almost reached full growth at the time of initiation of these exposures, whereas males far from their peak growth were used, the different growth rates of the two sexes are not believed to be attributable to the exposure.

The control rats grew about 9 per cent faster than those exposed to a concentration of 470 mg/m^3, whereas those exposed to 77 mg/m^3 gained the same amount or slightly more than their controls for the same period of time. The mature dogs showed slight fluctuations in weight but no general trend in either direction.

There were no changes between the sugar or albumin content of the urine of the exposed rats and the controls. However, there were considerable differences in the pH, volume, and creatinine coefficient, as shown in Table II. The changes were analyzed by the "t" test[12] and found

TABLE I

Exposures of Animals to Aerosols of Boron Oxide

Species	No.	Chamber size liters	Average concentration mg/m^3	Duration of exposure weeks	Particle size mass median diameter microns
Rat	70	1000	77	24	2.5
Rat	4	20	175	12	1.9
Rat	20	100	470	10	2.4
Dog	3	1000	57	23	2.4

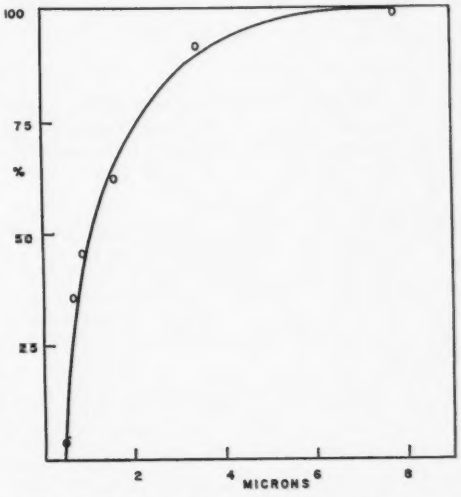

FIGURE 1. Particle size distribution (mean mass diameter in microns) of aerosols of boron oxide expressed as cumulative per cent by weight. Determined by use of a modified Rochester Cascade Impactor.

to be significantly different, with the following values of probability: volume $P < 5$ per cent, pH and creatinine coefficient $P < 1$ per cent. The formation of boric acid by hydration in the body probably caused the greater acidity of the urine of the exposed rats. The increased volume is undoubtedly accounted for by the known diuretic property of boric acid. The cause of increased creatinine excretion is not known. These values returned to normal one week after termination of the exposure.

Chemical analyses of six common blood constituents are given in Table III, for groups of rats exposed through twenty-four weeks to two concentrations of aerosols. There were no constant changes in either direction and no significant difference from the control values.

Sections of the lung, trachea, pancreas, thy-

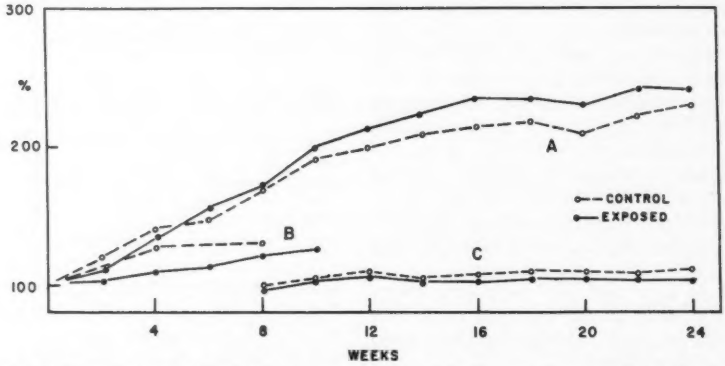

FIGURE 2. Comparison of weights of control and exposed rats (expressed as per cent of original weight). A. Males exposed to 77 mg/m³ and their controls. B. Females exposed to 470 mg/m³ and their controls. C. Females exposed to 77 mg/m³ and their controls.

TABLE II

The pH, Volume, and Creatinine Coefficient for Urine of Control and of Exposed Rats (concentration 77 mg/m³)

Weeks of exposure	pH		Volume ml/kg/day		Creatinine coefficient mg/kg/day	
	Exposed	Control	Exposed	Control	Exposed	Control
4	8.66	8.94	30	12	14.7	2.2
6	—	—	33	43	9.3	4.0
8	8.30	8.85	44	20	13.9	1.6
10	—	—	52	24	18.1	3.6
12	—	—	41	21	17.2	3.8
14	—	—	55	22	12.1	10.8
16	8.24	8.94	28	13	18.1	7.6
18	8.16	8.78	23	11	16.3	11.8
20	7.38	9.05	17	11	17.9	11.2
22	8.24	8.90	24	17	14.9	7.2
Average	8.16	8.91	34.7	19.4	15.3	6.4

roid, adrenal, eye, femur, rib, bone marrow, liver, heart, spleen, kidney, brain, stomach, intestine, ovary, testis, lymph node, and muscle were examined histologically for evidence of pathology. No differences were noted between the tissues of the exposed and control animals. There were no signs of pneumoconiosis.

Samples of the above tissues and controls were dissolved in 20 per cent sodium hydroxide and analyzed spectrographically for boron content. The rats had been exposed for six weeks to a concentration of 77 mg/m³ of B_2O_3. There was no boron found in any of the samples. Standard solutions of boron oxide in water were analyzed and showed that the method could detect a minimum of 2.5 micrograms of boron per milliliter.

TABLE III

Chemical Analyses of the Blood of Rats Exposed to B_2O_3 Aerosols

Time of exposure weeks	Sugar mg %	Lactic acid mg %	Protein gm %	Inorganic phosphate mg %	Creatinine mg %	Cholesterol mg %
Females exposed to 470 mg/m³						
2	119	29	5.9	10.5	1.0	—
4	53	50	5.5	8.4	1.2	—
6	52	52	7.5	—	—	—
8	78	30	6.6	4.7	—	—
10	55	60	7.4	5.1	—	—
Ave.	71	44	6.6	7.2	1.1	—
Controls						
Ave.	68	52	6.2	8.6	0.9	—
Males exposed to 77 mg/m³						
2	116	37	7.2	5.6	—	—
4	120	14	9.6	4.4	1.9	—
6	87	47	5.8	4.2	0.8	—
8	80	39	6.5	5.0	1.2	—
10	120	32	6.2	4.4	0.9	—
12	59	28	4.5	5.4	0.8	—
14	88	29	7.3	5.1	0.9	—
16	104	30	6.7	5.2	1.0	83
18	86	27	6.8	4.4	0.6	—
20	161	13	7.4	4.6	1.0	91
22	138	37	6.8	5.1	0.8	121
24	82	55	7.5	4.7	0.5	127
Ave.	103	32	6.8	4.8	0.94	101
Controls						
Ave.	104	37	6.8	5.5	1.04	100

The method would have detected 0.01 per cent of boron in the lung sample analyzed and one-third that amount in the other tissues. However, the rats were in metabolism cages for 60 hours after exposure and before sacrifice. If boron had been present it is possible that it was eliminated during that time.

The urine of control and exposed rats was analyzed by spectrographic methods for boron. The data show that considerable amounts of boron were excreted by the exposed rats and averaged 11.90 mg/kg/day. The controls excreted 0.24 mg/kg/day, or about ten micrograms per milliliter. The data are presented in Table IV.

The per cent of body weight of heart, lung, liver, and kidney from five rats exposed to the aerosol for 20 weeks was compared with control rats. The differences were not significant.

TABLE IV

Boron Content of Urine of Control and Rats Exposed to Aerosols of Boron Oxide, the concentrations averaged 77 mg/m³

Weeks of exposure	Urinary boron content mg/kg/day	
	Controls	Exposed
2	—	16.6
4	0.7	12.3
6	0.2	7.4
8	0.3	1.9
10	0.2	5.5
12	0.1	23.2
14	0.2	2.8
16	0.1	20.7
18	0.1	20.7
20	0.3	7.0
22	0.2	12.7
Average.....	0.24	11.90
After exposure		
1	0.3	0.3
2	0.5	0.9

TABLE V

Fragility of Femurs of Control and Rats Exposed to An Aerosol of B_2O_3

Group	No.	Least diameter mm.	Fracture weight kg.	Fracture weight / Least Diam. Average	Standard deviation
Controls...	14	2.68	6.6	2.43	0.69
Exposed*..	8	2.70	6.2	2.30	0.87

* Averages of groups that had been exposed for six and ten weeks to a concentration of 470 mg/m³

The fragility of rat femurs, as measured by the breaking point, is shown in Table V. The ratio of the fracture weight (kg) to the least diameter (mm) was taken as the index for comparison of bone fragilities. There was no significant dif-

TABLE VI

Hematological Values for Dogs, Pre-exposure, During Exposure to B_2O_3 Aerosols, and Controls

Dog No.	RBC × 10^6	WBC × 10^3	Hemoglobin gm.%	Eosinophils %	Stab (Band) %	Polymorphs %	Lymphocyte %	Hematocrit %
Pre-exposure (average of three samples from each dog)								
1	7.89	17.6	15.0	8.5	6.0	45	33	50
223	7.26	9.1	13.9	2.0	1.0	62	34	45
11	8.61	11.3	16.1	1.0	2.5	65	30	55
0	6.92	14.1	13.8	9.5	9.5	73	13	45
During exposure (average of 11 samples from each dog)								
1	7.03	21.3	15.7	9.3	11.1	41	38	42
223	7.39	12.9	16.6	3.0	3.0	55	29	44
11	8.20	15.2	18.7	3.1	3.1	53	36	49
Controls*								
0	7.95	11.5	18.2	10.1	5.2	60	21	48

* Average of 11 samples taken at the same time as those from exposed animals.

TABLE VII

Chemical Analyses of the Blood of Control and Dogs Exposed to B_2O_3 Aerosol

Sugar mg %	Protein gm %	Lactic acid mg %	Inorganic phosphorous mg %	Creatinine mg %	Cholesterol mg %
Before exposure					
86(9)*	6.9(8)	10(9)	3.3(3)	1.6(7)	n.d.**
Control					
88(3)	6.8(3)	20(3)	2.9(1)	1.8(3)	n.d.
During exposure					
101(30)	7.8(31)	19(30)	3.4(30)	1.0(25)	256(11)
Control					
109(9)	7.3(9)	20(8)	3.3(9)	1.1(7)	251(3)

* Figures in parentheses denote number of samples averaged.
** Not determined.

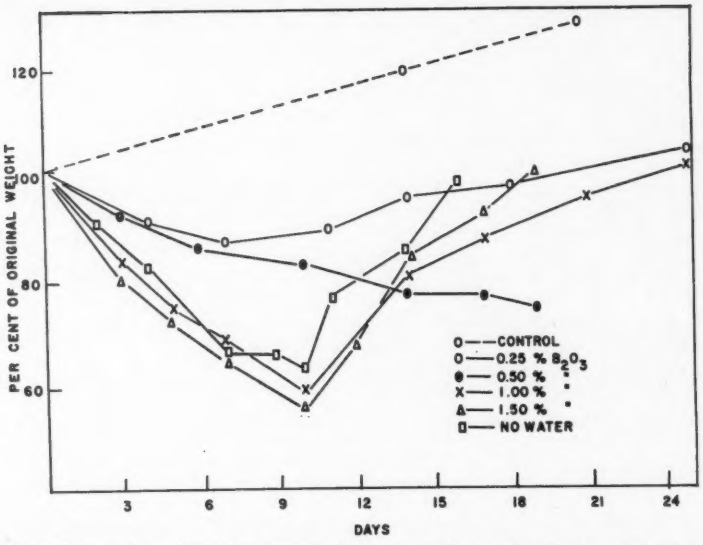

FIGURE 3. Comparison of weights of control and exposed rats given water containing boron oxide (expressed as per cent of original weight). On the tenth day tap water was given to those groups represented by the lower three curves.

ference between the controls and those exposed to the aerosol, as shown by the "t" test.[15]

Roentgenograms of control rats and those exposed to 77 mg/m³ of boron oxide for ten weeks showed no detectable effects due to B_2O_3.

There was a slight and probably insignificant rise in the leucocyte counts of the exposed dogs that may suggest a slight response to B_2O_3 aerosol. There were no other changes, except for the usual fluctuations, and no significant difference from the control (Table VI).

In Table VII are given the results of the chemical analyses of some constituents of the dog blood. There were no changes from the pre-exposure values nor from those of the control dog. Sulfobromophthalein retention tests, for liver damage, were also negative as compared to the control.

The weight loss of the rats (Figure 3) that were given 1.0 or 1.5 per cent of boron oxide in water was rapid for ten days, and closely approximated the weight loss of a similar group of rats given food but no water. At the end of ten days, all of the above rats were in very poor condition, having lost about 40 per cent of their original weight. This loss undoubtedly was caused by refusal of the animals to accept the water containing the boron oxide, and subsequent starvation, by abstaining from food also.

Tap water was given to the rats on the tenth day and resulted in a rapid and steady gain in weight. The two groups fed on 0.50 and 0.25 per cent B_2O_3 water also lost weight, the former to 73 per cent of its control value in 19 days, while the latter group slowly regained weight after a small initial loss.

Ten rats were given a daily intragastric intubation of a ten per cent slurry of boron oxide in water for three weeks. The dose given daily was approximately 0.5 gram per kilogram. The test rats, and a control group of ten, were weighed daily. At the termination of the period the intubated rats showed no toxic signs and had gained practically the same amount of weight as the controls, 121, and 122 per cent respectively.

Topical application of boron oxide dust to the clipped backs of rabbits produced erythema that persisted for two to three days. Similarly, almost immediate conjunctivitis resulted when the dust was applied to the eyes. These reactions were undoubtedly caused by the exothermic reaction of hydration to boric acid.

Summary

Rats have been exposed to aerosols of boron oxide in dynamic exposure chambers for as long as 24 weeks. The highest concentration was 470 mg/m³ for a period of ten weeks.

Dogs have been exposed to a concentration of 57 mg/m³ for 23 weeks.

No toxic signs have been shown in any of the animals. Chemical analyses of dog blood and rat blood and urine have shown no changes from control values, except for an increased urinary excretion of creatinine in rats, lower pH, increased volume, and increased boron content of rat urine.

No changes have been found as a result of aerosol exposures in the following: rat tissues and organs, bone fragility, roentgenograms of rat bones, hematology of dog blood, sulfobromophthalein retention, and rat organ weights.

Rats refused to drink water containing 1.0 or 1.5 per cent boron oxide and therefore lost weight. Intragastric intubation of a ten per cent slurry of boron oxide into rats did not retard their growth rate nor cause any noticeable effect.

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Seventh Detroit Anachem Conference

THE SEVENTH DETROIT ANACHEM CONFERENCE will be held at the McGregor Memorial Conference Center at Wayne State University on October 26, 27, and 28, 1959. The program includes both authoritative review papers and contributed papers covering recent advances in analytical chemistry. Presentations will be made by outstanding men from industry and universities. Features of the program are the Anachem Award Session in honor of G. Frederick Smith, and a Conference Dinner with John C. Bailar, President of the American Chemical Society, as guest speaker.

Exhibits of analytical instruments and equipment will be displayed in a spacious exhibit area adjacent to the main auditorium. Thirty-three leading suppliers will be represented.

For the convenience of those attending, luncheons and the Conference dinner will be served in McGregor Memorial dining room. Reservations for meals must be made in advance. For complete information on registration, program and accomodations write to J. W. Compton, Research Department, Wyandotte Chemicals Corporation, Wyandotte, Michigan.

Hearing Loss Related to Non-Steady Noise Exposures*

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HEARING LOSS related to noisy occupations has long been recognized as an entity having been first noted in millers by Ramazzini. Kryter[1] notes that Fosbrooke speaks of "old" and "modern" discussants on the subject in 1831. The last two decades have shown an accelerating upsurge in interest in the occupational deafness problem. Studies by McCoy,[2] Larsen,[3] Gardner,[4] Johnston[5] and others have confirmed the presence of hearing loss for high frequencies after exposure to noises.

Only recently have attempts been made to relate hearing loss to industrial noise exposures in any quantitative way. These studies conducted by the American Standards Association[6] and Rosenwinkel and Stewart[7] were limited to steady-state noises which could easily be described by sound level meter-octave band equipment.

Limitations of conventional noise measurement equipment led Stewart to design a completely new type of device which integrates sound energy over finite time periods. The principles and details of the design of this instrument were presented by Stewart to AIHA at the 1954 Industrial Health Conference held in Chicago in April of that year. This instrument integrates over a five second interval the logarithm of all of the noise between 300 and 5000 cycles per second above a 60 db threshold sensed through a weighted microphone.

The present study is a tentative exploration of the relationship which may exist between hearing loss and industrial noises measured with the Stewart noise dosimeter.

In order to secure a sample population of a size large enough to allow statistical analysis while still meeting the stated criteria for selection, it was necessary that a large industrial establishment be available. It was fortunate that a suitable plant gave its support to this project. This facility employs over 25,000 persons and the firm's policy of upgrading its employees from unskilled starting jobs to more skilled positions has brought about a rather unusual condition in which a majority of the employees have spent their entire industrial careers at this one plant. The plant consists of several large machine shops, several fabricating divisions for major sub-assemblies, final product assembly divisions, and a large foundry.

The test population was selected from the workers at seven different locations in the plant. These locations encompassed a wide range of noise levels and provided the means for an investigation into the existence of a dose-hearing loss relationship. The characteristics of the noise in most locations were such that meaningful sound level meter octave band measurements were not possible.

The population exposed to these noises consisted of male employees of less than 35 years of age who had up to 10 years of exposure. Workers with exposures limited to only one type of noise were not available in this plant, therefore, a compromise criterion was adopted for subject selection which would allow exposure to other noises for not more than 20 per cent of an industrial career. This 20 per cent exposure, furthermore, must have been to noises which were judged to be no more severe than the present environment. Workers whose audiograms indicated a severe conductive hearing loss were eliminated from the study. The distribution of the test population among the seven locations is shown on Table I.

The test environment and audiometric techniques employed in this survey approached the best clinical practices. For a detailed description of these procedures, reference is made to the published report of an earlier investigation by the authors.[7]

The study demanded a quantitative measurement of the noise and the environment to serve

* The opinions expressed in this article are those of the authors, and are not necessarily those of the Department of the Navy.

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as the basis for a relative noise dose rate from which the cumulative noise doses for each test subject could be calculated. A sampling technique randomized within certain strata was devised as the best means of arriving at such a measurement. For practical reasons the period of a week was selected as a major subdivision of time over which sampling was to be performed. In order to account for possible temperal variations in the noise level at the seven sampling locations, the week was divided into halves and the days in thirds. It seemed likely that the pace of production (and the amount of noise generated) might undergo cyclical changes during the day and week and that such variations must be recognized in computing relative noise dose rates for each of the seven locations from which the test population was drawn. Thus, the final plan was to divide the week into six cells (the week into halves and the work days into thirds) with each of the seven locations to be sampled once in each cell, thereby giving a total of 42 environmental samples each week, six for each location.

The individual samples consisted of 120 five-second integrations of the noise dose meter for a total sampling time of ten minutes. The specific point at which a sample was to be obtained at each location was selected by a random choice of an individual out of all the workers included in the study at this location. The order in which the locations were sampled was also randomized within each of the six cells in a week. The technique of actually obtaining the readings which constitute the environmental sample was quite simple once the actual spot for sampling was chosen.

The meter itself is easily carried and simple to operate. The problems of microphone position, meter range, selection, and so forth are no different from those encountered in most sound measuring instruments.

During sampling the calibration of the instrument was maintained to give a reading of 80 units when placed in a sound field of 80 db re 0.0002 dynes/cm^2 at 1,000 cycles per second.

During the sampling of the noise environment a total of twelve ten-minute samples were obtained on each of the seven locations. Since each sample consisted of 120 five-second integrations, a total of 1,440 meter readings were available to estimate the relative noise dose rate at each location.

The means of the meter readings are actually logarithmic functions since the noise dose meter at one stage in its computer circuitry takes the common logarithm of the noise function sensed by the weighted microphone. It is necessary for the instrument to take the common logarithm of the noise function in order to reduce the range of the summations of the noise energy to a manageable size dictated by components of the electronic circuit. To restore the dose readings to their proper relationships, the antilogarithms of the meter readings were needed. Again, to keep the numbers thus obtained down to practical size the mean meter readings were first divided by 100 before the antilogarithm was taken. The authors have named this quantity the relative noise dose rate.

TABLE I

Data on Test Population and Noise Exposures

Location	Type of Exposure	Number	Mean Meter Reading	Relative Noise Dose Rate
1	Machine Operators	32	87.70	7.53
2	Drill Operators	9	86.74	7.36
3	Product Assemblers	5	88.07	7.60
4	Arc Welders	12	92.33	8.38
5	Product Assemblers	16	85.44	7.15
6	Pneumatic Chippers	7	106.71	11.65
7	Arc Welders	9	95.71	9.05

To calculate the estimated cumulative noise dose, the antilogarithm obtained for any location was multiplied by the number of months of exposure for all individuals within that location. For example, a pneumatic chipper working in location #6 for 100 months where the relative noise dose rate is 11.65 would have a calculated cumulative noise dose of 1,165 dose units.

No specific physical definition of the dose unit is tendered at this time. The particular range of values in this study from 81 to just over 1400, evolved from the manner in which the instrument was calibrated, the derivation of the relative noise dose rate, and the length of exposures of the test subjects included in the survey. Thus, the range used expresses only the relative insult on hearing, weighted by the severity and length of exposure and not a quantitative measurement of noise energy delivered over the time of the study.

A table of the mean meter reading, the relative noise dose rate, number of men, and type of exposure for each of the seven locations is presented in Table I.

In the analysis of these data, the object has been to describe statistically the relationship which exists between the observed hearing loss in a test group and the calculated cumulative noise dose of the individuals who make up that group. This analysis is based on an assumed linear relationship between the variables of the

TABLE II

Table of Statistical Values of the Relationship of Observed Hearing Loss with Cumulative Noise Dose

Audiometric test frequency in cps	Regression coefficient	Correlaton coefficient	Standard error of estimate in db	t
2000	.014	.31	11.6	10.0
3000	.038	.46	19.2	17.8
4000	.037	.49	17.0	21.7
6000	.030	.38	19.2	06.7

hearing loss and the cumulative noise dose. To describe this relationship it is necessary to determine the regression coefficient of a line which best fits the data obtained. In addition to this, a correlation coefficient is calculated to describe the intensity of the relationship which exists between the two variables. The statistical significance of the numerical value of a slope may be determined by the value of t which is derived for that slope if one applies the assumption that the true slope is zero. A table of the numerical values of the regression coefficient, the correlation coefficient, the variance of the hearing losses about the mean regression line and the values of t obtained for each audiometric test frequency are presented in Table II. These data yield evidence of a significant relationship between hearing loss and calculated cumulative noise dose. If the assumption of no linear relationship between the two variables is tested, the probability of obtaining the values of b and r as a matter of chance for the four frequencies considered would be less than 5 in 100. The significant values for t and r at the 95 per cent levels of confidence, for the number of observations in this analysis, are approximately 1.98 and 0.21, respectively.

Considering the individual audiometric test frequencies it can be seen that the values of b and r are at or near a maximum for 4,000 cycles per second. Since it is generally agreed that the ear is most susceptible to acoustic trauma at 4,000 cycles per second, this supporting evidence of the steepest slope and strongest association between the variables is suggestive that the calculated cumulative noise dose derived from measurements with the Stewart noise dose meter explains a part of the hearing loss as measured in this survey. In addition to the values of b and r it is noted that the value of t is greatest for 4,000 cycles.

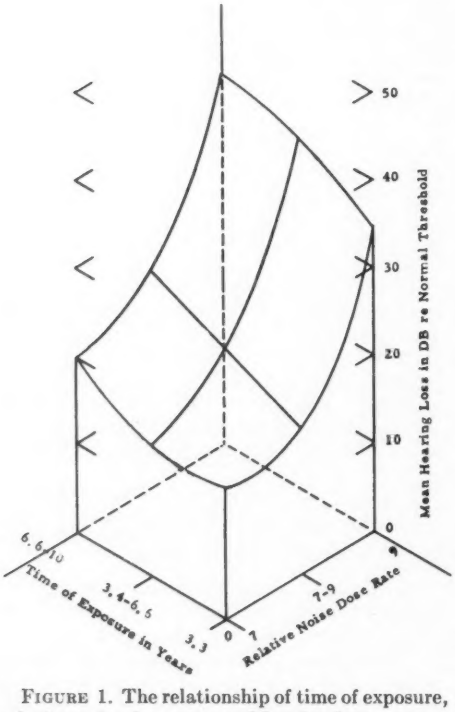

FIGURE 1. The relationship of time of exposure, relative noise dose rate, and hearing loss.

Figure 1, presents a three dimensional characterization of the relationship of the three variables, hearing loss, relative noise dose rate, and time of exposure. This figure indicates that both the rate of dosage and the time of exposure are probably of equal importance in explaining the observed hearing losses. The losses observed are the result of some complex function of time, rate, and the interaction of the two rather than a simple product such as is assumed in this study. The shape of the hearing loss surface provides an indication of the nature of this function.

A disappointing result of this analysis is that the relationship between observed hearing loss and calculated cumulative noise dose is not more intense. Some causes of this weakness are inherent in factors which contribute to the variance of hearing loss about the mean regression line. Among these are the following: (a) hearing acuity as such is a biological attribute which is subject to variation among individuals and is thus distributed about a mean value even for persons of like characteristics; (b) susceptibility to acoustic trauma is subject to variation among individuals; (c) some of the observed hearing loss is due to factors not accounted for in the calculated cumulative noise dose, such as unrecognized ear pathology, non-occupational or

military noise exposures, noise exposure on jobs other than the present one, and so forth; (d) inherent errors in the technique of measuring hearing loss.

Conclusions

(1) A significant linear relationship exists between observed hearing losses in the test population and a calculated cumulative noise dose derived from environmental noise measurements conducted with the Stewart noise dose meter. This relationship exists at all frequencies of 2000 cps and over, but is most intense at 4000 cps, the frequency most susceptible to acoustic trauma.

(2) The described relationship cannot be used as an estimator of hearing loss for individuals exposed to noise due to the wide variance of observed hearing losses about the mean.

(3) The intensities of the relationship between observed hearing loss and each of the two factors, time of exposure and relative noise dose rate, are about equal.

(4) The present data are inadequate to describe the precise relationship between observed hearing losses and exposure to noise.

Summary

This study was a first investigation into the possible existence of a relationship between noise as measured with an integrating type meter and hearing loss. The noise to which the persons were exposed was widely fluctuating and not sensibly measurable with the conventional types of noise measuring equipment. As a first approximation, the existence of the relationship has been investigated on a linear basis. The relationship describes the distribution of hearing loss in a population and must not be applied as a predictor of hearing loss in individuals. The true nature of the relationship between hearing loss and noise exposure cannot be discerned from these data, however, it appears to be some function of time, rate, and an interaction between the two.

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Action of Chlorinated Hydrocarbons

AT THE FEDERATION OF AMERICAN SOCIETIES for Experimental Biology, D. N. Calvert and T. M. Brody of the University of Michigan Medical Center reported a 2½ year study of the toxic effects of carbon tetrachloride. They reported that this compound and probably other chlorinated hydrocarbons are not poisonous but that they trigger a massive and prolonged discharge of adrenalin within the body. The resulting overload of adrenalin can severely damage the liver and prove fatal. The wholesale discharge of adrenalin somehow breaks down the body's stored fat and fills the bloodstream with fatty acids. At the same time, it impairs the flow of blood to the liver so that the organ cannot function normally. This chain reaction can be interrupted by blocking the signals from the brain and test animals show no ill effects from otherwise near-fatal doses of carbon tetrachloride.

Administrative Experience with Occupational Overexposure to Radiation*

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Introduction

MOST OF THE information on occupational overexposure to radiation has come from the Atomic Energy Commission based on its own experience, those of its contractor-operated installations and those of its licensees. The material deals largely with criticality accidents. Very little information on overexposure has been published by state and local agencies. Certain incidents, such as overexposure resulting from rupture of sealed sources (Texas) and from lost or stolen sources have been publicized in various news media often, unfortunately, before all the facts have been fully evaluated.

The purpose of this paper is to present a state agency's data on occupational overexposure, highlighting the administrative experience with this problem.

Data

Since December, 1955, when Industrial Code Rule 38 went into effect, 19 instances of excessive radiation exposure have been reported to the New York State Labor Department and have been investigated by the Division of Industrial Hygiene. The Code defines overexposure as a known or suspected dose of more than 3 rems of external radiation or an equivalent internal exposure from inhaled or ingested radioactive material over a 13 week period.

Reports of overexposures are based on readings of film badges, pocket dosimeters, surveys and air sampling. In eight of the 19 instances, the reported excessive radiation readings did not involve an overexposure of workers. Table I is an analysis of these eight situations. The responsible factors, such as faulty dosimetry techniques and inaccurate film badge analysis, and the corrective measures instituted are noted in the Table and will be discussed subsequently.

Of the eleven situations where actual exposures of personnel occurred, four were chronic overexposures, four were acute overexposures to external radiation and three were acute overexposures to internal radiation.

Table II summarizes the data of the four instances of chronic overexposure to personnel. Three of the four situations occurred in radium processing, and the other one occurred in field radiography during the handling of Cobalt-60 and Iridium-192. In this total, 31 workers were involved and the estimated dose ranged from 7-60 rems per quarter-year, as based on dosimetry and on hazard survey measurements of the dose rates and durations of exposure. Four of the workers who handled radium showed a radiodermatitis and one had evidence of excessive absorption of radium and polonium in his tissues. The duration of exposure for the radium workers was 15–25 years and 5–10 years for the radiographers.

Table III shows the pertinent data on the four reported instances of acute overexposure of personnel to external radiation. In view of the fact that there were acute accidental exposures, only the estimated total dosage for the incident is shown. The figures given exceed the permissible quarterly limit. Of the 16 workers involved, radiation burns were sustained by three. These were exposed to localized high radiation doses ranging from 300 to 1100 rems, based on the measured dose rates and estimated duration of exposure.

The data on acute internal overexposure of personnel are tabulated in Table IV. The estimated equivalent dose sustained by the 22 workers ranged from 0.2–10 rems, calculated on the basis of an air concentration which would produce a dosage of 300 mrem in a critical organ over a 40 hour workweek. The positive biological data disclosed retention of about 10 per cent of the maximum permissible Strontium-90 body burden in one employee, absorption of tritium and retention estimated at less than 2 per cent of maximum permissible body burden in another employee, and in four additional employees, absorption of uranium and thorium with no evidence of significant retention.

Table V presents a summary of the factors responsible for the overexposures and the number of instances in which each factor was involved.

* Presented at the Twentieth Annual Meeting of the American Industrial Hygiene Association, April 25-May 1, 1959, Chicago, Illinois.

TABLE I

Instances of Reported Excessive Radiation Readings not Involving Overexposure of Personnel, January 1956–December 1958

No.	Source	Average exposures to employees in rems/week	Reported reading in rems/week	Responsible factors	Corrective measures instituted
1	X-ray Thickness Gauge, 70 KVP	<0.01	8.5	Lack of control over issuance, use and return of dosimeters	Control by management over storage, issuance, use and return of dosimeters tightened
2	Medical X-ray, 90 KVP	0.01–.05	14.5	Employee wore film badge while his own arm was being x-rayed	Same as (1)
3	Industrial X-ray Unit, 250 KVP	0.05–.075	50	Employee carelessly left badge in high radiation area while unit was in operation	Same as (1)
4	Beta-ray Gauge, Sr 90	<0.01	0.5–4.0	Rupture of metal shield for Sr 90 source	1. Contaminated area evacuated and decontaminated 2. Source removed for repair
5	Beta-ray Gauges, TI 204, Sr 90, Cs 137	0.025–0.1	5.3	Error made by film badge service in reading dosimeter	Film badge service revised procedures to attain greater accuracy in reading dosimeters
6	Beta-ray Gauges, Sr 90	0.03–0.125	66.8	Same as (1)	Same as (1)
7	Radioactive Static Eliminators, Ra-226	0.04–0.15	10	Employee exposed badge to source to check reliability of dosimeter	Same as (1)
8	Radioactive Luminous Compound, Ra-226	0.1–0.3	0.5–2.5	Storage of dosimeters in high radiation area	Shielded storage facilities for film badges provided

TABLE II

Instances of Chronic Overexposure of Personnel to Radiation, January 1956–December 1958

No.	Source of overexposure	Number of employees exposed	Estimated dose in rems/13 weeks	Positive biological data	Corrected measures instituted
1	Employees processing radium and daughter products were exposed to whole body gamma radiation and airborne radon concentrations	11	8–15	High breath radon, excessive absorption of radium and polonium in tissues—1 employee. Radiodermatitis to hands—2 employees	1. Radiation safety consultant retained. 2. Surveys and air sampling performed periodically. 3. Personnel monitoring introduced. 4. Working hours reduced. 5. Periodic breath radon tests performed.
2	Same as (1)	10	7–14	Radiodermatitis to hands—one employee	1. Adequate shielding and ventilation provided. 2. Surveys and air sampling performed periodically. 3. Personnel monitoring introduced.
3	Employees processing radium and daughter products were exposed to excessive gamma radiation, particularly to hands	5	25–50	Radiodermatitis to hands—two employees	Exposed personnel rotated.
4	Radiographers handled Cobalt 60 and Iridium 192 pills manually	5	30–60	None	Devices for remote handling of pills provided.

TABLE III

Instances of Acute Overexposure of Personnel to External Radiation, January 1956–December 1958

No.	Source of overexposure	Number of employees exposed	Estimated dose in rems	Positive biological data	Corrective measures instituted
5	Change of position of 250 KVP X-ray tube during operational check exposed lower extremities of employees to unshielded beam.	3	5–25	None	1. Qualified radiation safety officer appointed. 2. Completely shielded testing facility provided. 3. Surveys performed periodically.
6	Failure of an interlocking device on a 50 KVP cabinet-type industrial X-ray unit which employee used as "on-off" switch.	1	300–800	Radiation burns to hands of one employee	1. Interlock repaired and checked regularly 2. Unsafe practice of using interlock as "on-off" switch discontinued. 3. Radiation warning signals installed.
7	Employees failed to restore shielding for 140 KVP microwave generator after making circuit adjustments, thereby exposing head and trunk to X-rays.	8	5–30	None	1. Use of equipment restricted to trained personnel. 2. Surveys performed regularly. 3. Radiation warning signals installed.
8	Employees, unaware that a 1.5 MEV particle accelerator was energized, entered high radiation area.	4	400–1100	Radiation burns to face and hands—Two employees.	1. Interlock for auxiliary voltage source provided. 2. Use of equipment restricted to properly instructed personnel.

TABLE IV

Instances of Acute Overexposure of Personnel to Internal Radiation January 1956–December 1958

No.	Source of overexposure	Number of employees exposed	Estimated dose in rems	Positive biological data	Corrective measures instituted
9	Spillage of highly concentrated Sr 90 solution	6	0.2–4.0	Retention of about 10% of maximum permissible Sr 90 body burden—1 employee	1. Premises decontaminated 2. Supervision over operations tightened. 3. Radiation safety manual prepared and issued to exposed employees.
10	Accidental release of radioactive tritium gas from container	7	2–10	Absorption of tritium—1 employee. Retention estimated at less than 2% of maximum permissible body burden	1. Apparatus re-designed. 2. Adequate ventilation provided. 3. Air sampling and bio-assay program initiated.
11	Inhalation of radioactive uranium and thorium dust associated with explosion of pyrophoric scrap material	9	0.2–5.0	Absorption of uranium and thorium—4 employees. No evidence of retention	1. Operations isolated 2. Remote-control devices installed

TABLE V

Causal Factors in Overexposures

Factor	Number of instances involved
1. Lack of Proper Supervision	8
2. Lack of Awareness of Hazard	9
3. Poor Design and Layout of Installation	2
4. Failure to Provide Protective Devices	5
5. Lack of Knowledge of Control Measures	2
6. Carelessness of Operating Personnel	4

Discussion

The 19 incidents of radiation overexposure based on readings of film badges, pocket dosimeters, air sampling and other radiation-measuring devices reported to New York State between 1956 and 1958 demonstrate the need for stricter adherence to the principles of radiation control as contained in Industrial Code Rule 38 and the need for more reliable dosimetry. Since we cannot always determine by clinical and biological methods whether an actual overexposure

to personnel has occurred, it is particularly important that greater care be taken to insure that dosimeters are not exposed to radiations which do not reflect actual exposure of employees.

Of the eight instances of excessive readings shown in Table I, six could have been prevented by adequate instruction and supervision in the use, return and storage of film badges. For example, in number 6, the film badges were issued in a haphazard manner, used without supervision, returned for assaying at the discretion of the employee, and left in radiation areas when not in use. It would be difficult to define the specific factor responsible for the high film badge reading, but on the basis of the dose rate in the work area it is presumed that the employee could not have sustained such an overexposure. On the other hand, in number 8, the high readings could definitely be traced to improper storage of the film badges in high radiation areas. In number 5, the abnormal reading was the result of a faulty processing technique by a commercial film badge service. This instance illustrates the need for the periodic spot-checking of film badge services for reliability. About four years ago, the Atomic Energy Commission recognized this need and asked the National Bureau of Standards to check the accuracy of film badge readings by commerical services. More recently, similar tests were performed by the New York City Health Department and, as a result, the Department made available a list of approved film badge services.

In number 4, after an adjustment of a rolling and calendering machine had been made, the workmen failed to replace the safety bolt when returning the beta-ray gauge head to the machine. With resumption the operations, the unattached head containing the source fell to the floor, rupturing the metal foil which served as the shield for the source, and Strontium-90 was released to the atmosphere in toxic concentrations. In this instance, if a precautionary check had been made prior to resuming operations, this accident would not have occurred. Fortunately, prompt evacuation of the personnel and the immediate roping off of the contaminated area prevented an overexposure of the workers.

In four instances of chronic overexposure (Table II), three occurred in the radium-processing industry. Of the 26 workers chronically exposed to radium, biological effects were observed in five. Since these effects require sufficient time to be manifested, it is probable that the overexposure existed prior to the promulgation of the code. These five workers were exposed to higher concentrations and intensities over a longer period of time than were the others. It is noteworthy that since the corrective measures were instituted, no new cases of overexposure or injury of personnel have been reported. The shorter length of employment and the brevity of the exposures of the industrial radiographers, as compared to the radium workers, probably accounts for the absence of clinical effects in this group.

Acute overexposure to external radiation (Table III) involved 16 employees of whom three sustained radiation burns. These occurred where the estimated dose ranged from 300 to 1100 rems. Of the three injuries sustained, two occurred in highly competent professional people who were exposed to excessive radiation from a machine not regarded as capable of emitting high radiation intensities under the conditions of use. In the three cases, failure to provide and maintain an interlocking device was an important factor in causing the overexposure.

The instances of internal overexposure to personnel (Table IV) illustrate other situations of employee carelessness and lack of appreciation of the hazard. The use of breakable glass containers for large quantities of gaseous radioactive material (number 10), and the failure to notify supervisory personnel promptly of an abnormally high concentration of Strontium-90 in the workroom atmosphere exemplify this. As is seen in Table IV, the two employees in instances 9 and 10 who showed retention of radioactive material were those who actually handled the sources and who were subjected to the most intense atmospheric concentrations of Strontium-90 and Tritium. It is significant that among the causative factors in the 11 cases of personnel overexposure as listed in Table V, the most frequent were lack of proper supervision and lack of awareness of the hazard.

In general our experience has shown that the extent of industrial overexposure to radiation in New York State is relatively low. In the 11 reported situations of overexposure to personnel, 14 employees of a total of 69, showed either clinical effects, or, through bio-assay, evidence of excessive absorption of radioactive material. On the basis of an estimated 5000 employees who are engaged in work with radiation sources in New York State, this represents an incidence of 1.4 per cent for radiation overexposure over the three-year period from 1956–1958. The incidence of observable injuries is 0.3 per cent. In both instances, human error was a contributory factor to the occurrence of overexposure and injury.

Preliminary results of a state-wide survey of radiation installations presently being conducted by the Division of Industrial Hygiene indicate that the exposure of more than 90 per cent of radiation workers does not exceed 50 mrem/week. This demonstrates the feasibility of maintaining safe operation within prescribed exposure limits for practically all sources. Similar findings and conclusions have been reached by the Atomic Energy Commission and industries following evaluation of their exposure data.

Summary and Conclusions

Nineteen instances of radiation overexposure in excess of the limits prescribed by Industrial Code Rule 38 have been reported to the New York State Department of Labor since December, 1955. In eight instances, the reported excessive readings were found to be invalid and may be ascribed, on the whole, to faulty dosimetry technique.

There were eleven instances of actual overexposure; four were chronic in nature, four were acute external overexposures, and three were acute internal overexposures. The acute overexposures resulted from accidents; the chronic overexposures were associated with routine everyday operations.

Ten employees showed positive biological effects such as radiation burns and/or abnormal retention of radioactive material. Injury to the hands was the effect most frequently observed among the chronically as well as the acutely exposed. These accounted for seven of the ten reported instances.

The causal factors most frequently involved in the overexposures were lack of supervision, lack of awareness of the hazard, and failure to provide or maintain appropriate protective devices.

In general, the data indicates that the incidence of radiation overexposure (1.4 per cent) and injury (0.3 per cent) in New York State is extremely low when compared to other major industrial hazards.

Industrial Nurses Conference

THE NEW YORK STATE Association of Industrial Nurses will hold its Eighth Annual Health Conference in New York City, November 6th through 8th, 1959, at the Commodore Hotel. The Greater New York Association of Industrial Nurses will be the hostess group with Miss Elizabeth Van Steenburgh of Johns Manville, Inc., as the Conference Chairman.

Educational programs are being arranged for Friday and Saturday. The annual business meeting will be held Saturday afternoon and new officers will be announced. At the banquet that evening Miss Fannie Hurst, noted author and lecturer, will be the speaker. At the Sunday breakfast meeting the speaker will be Miss Caroline Simon, Secretary of State for the State of New York.

Evaluation of Fallout Data*

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Introduction

AN ESSENTIAL part of the safety program in a laboratory manipulating radioactive materials is the routine determination of airborne activity if operations are compatible with the production of an inhalation hazard.[1] Laboratory air and air discharged from the laboratory should be monitored for radioactivity if there is any possibility of airborne contamination at hazardous levels.[2] When contamination of the air in the vicinity of a laboratory is a possibility, air surveys should be made to determine background levels of radioactivity before extensive operations are begun.[3]

The purposes of routine air sampling are thus threefold:

1. Protect employees
2. Public safety
3. Establish background information with respect to variations of radioactive concentrations in the environment unrelated to laboratory operations.

The National Committee on Radiation Protection and Measurement (NCRP) has established levels of maximum permissible concentrations (MPC) to serve as guides to safe operations and upper levels of exposures. Where many types of isotopes are in use, or for unknown mixtures of radioisotopes in air beyond areas that are under control of the installation responsible for the potential contamination, a provisional guide to values of permissible concentration of radioactive contaminants has been recommended, as follows:

10^{-9} μc/ml for beta or gamma emitters

5×10^{-12} μc/ml for alpha emitters.[4]

These values do not refer to natural backgrounds but to additions to the natural background as used by man.[5]

Because of the many uncertainties involved, the NCRP Subcommittee on Permissible Internal Dose recommends that every effort be made to keep concentrations of radioisotopes in air (and water and in the body) to a minimum. The goal should be no radioactive contamination of air (and water and of the body) if it can be accomplished with reasonable effort and expense. If such a goal cannot be attained, the average operating levels should be kept as far below recommended values as possible and not permitted to be above them for any extended period of time.[5]

Assays are complicated by the fact that MPCs may be less than naturally occurring decay products of radon and thoron.[5, 6] Methods used for the evaluation of activity in the presence of high concentrations of radon and thoron daughter products are described in the literature.[6, 7, 8, 9]

These methods suffer from the necessity of taking extended decay curves (24 hours) to evaluate the natural activity. More rapid evaluations may be possible by comparison methods utilizing meteorological and diurnal variables to estimate natural activity or by comparing samples taken from more than one sampling station.

Unfortunately, one source of man-made radiation can make evaluation difficult and, because of its unpredictability, always raises concern that airborne activity is the result of laboratory operations, i.e., fallout from nuclear detonations.

This report:

1. Describes currently used methods to evaluate radioactivity which may be a result of nuclear detonations.
2. Proposes a very simple method utilizing a specially prepared graph paper to characterize the activity from nuclear detonations.
3. Presents a method which can verify radioactivity from nuclear detonations without the extended time delay required for taking decay curves and minimizing the interference of other radioactive species.

Current Methods

The mixed fission product activity from a nuclear detonation can be approximated from a few minutes to a few years by

$$A = kt^{-1.2} \qquad (1)$$

where t = time after detonation
A = activity at time t.

If a decay curve is plotted on log-log paper, a

* Presented at the Twentieth Annual Meeting of the American Industrial Hygiene Association, April 30, 1959, Chicago, Illinois.

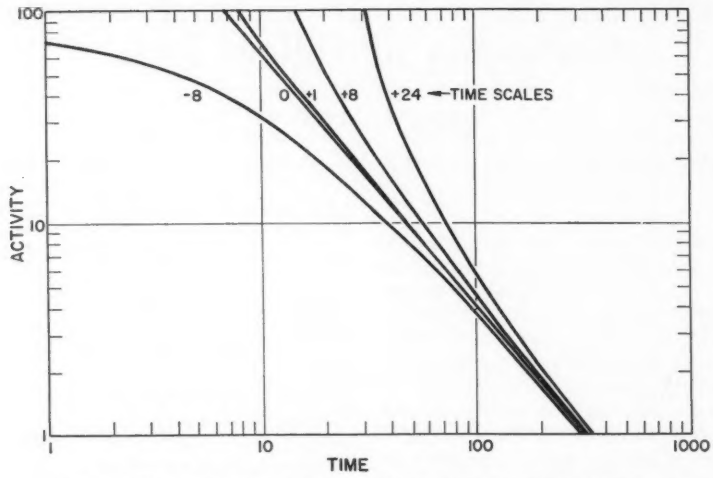

FIGURE 1. Decay of mixed fission products from a nuclear detonation.

straight line results. The activity can thus be predicted at any future time, but only if the exact time of detonation is known. If the detonation time is unknown and data is plotted using an incorrect origin for the time scale, the plot of the activity is not a straight line. Thus, if the time of detonation is unknown, not only is it difficult to determine whether foreign activity is from a detonation but, extrapolation for correction is difficult, if not impossible. A technique which has been used is to plot decay curves using a variety of time scales (i.e., zero's), to find the one which gives the best straight line and determine if a slope corresponding to the $t^{-1.2}$ is obtained. This is illustrated in Figure 1.

The time of detonation can be determined using Equation 1 and two measurements on the same sample. Assume the detonation took place at an unknown time interval t prior to the first measurement and the second measurement is made at a known time interval p after the first measurement. Then

$$A_1 = kt^{-1.2} \tag{2}$$

and

$$A_2 = k(t + p)^{-1.2} \tag{3}$$

whence

$$t = \frac{p}{(A_1/A_2)^{1/1.2} - 1} \tag{4}$$

It can be easily calculated from Equation 4 that when $A_1/A_2 = 2.3$, fission occurred at a time prior to the first measurement just equal to the time interval between measurements. Mixed fission product decay data is plotted on semi-log paper against the time of measurement (calendar date). The time interval for the activity to decay by a factor of 2.3 is measured and the time of fission determined. Some actual air sample decay data is shown in Figure 2 to illustrate the method.

A method described by Holter and Glascock involves plotting decay data on semi-log paper. An experimental sample curve is superimposed for the best fit to determine the date that fission occurred.[10]

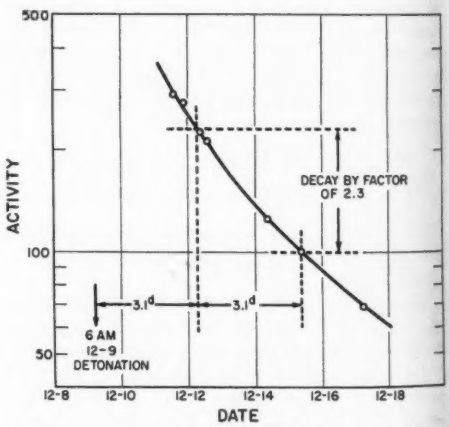

FIGURE 2. Dating a nuclear detonation by decay of mixed fission product activity.

Simple Method

These methods can be considerably simplified by a graphical transformation which adjusts nuclear detonation mixed fission product decay data to linear form. A decay curve is plotted on linear paper with time of measurement (calendar date) as the abscissa and the -1.2 root of the activity as the ordinate. Since, from Equation 1,

$$A^{-1/1.2} = k^{-1/1.2}t,$$

a straight line characterizes the decay data and also can be used to determine the activity at any time. Extrapolation to $A^{-1/1.2} = 0$, the birth date of the fission products sampled can be determined.

This method is further simplified by preparation of special graph paper with the ordinate labeled with the activity but ruled as the -1.2 root of the activity. The application of this paper is shown in Figure 3 plotting the same data used in the previous example. The straight line obtained identifies the activity as nuclear detonation debris and the time of fission was determined. Since the efficiency and geometry cancel out, any activity can be plotted by use of an appropriate factor to get all data on one graph.

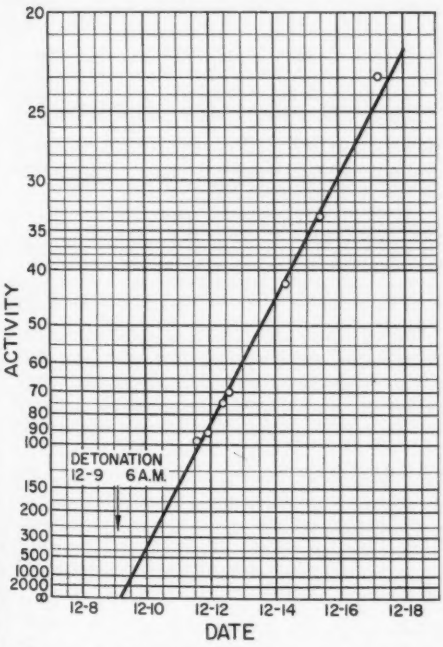

FIGURE 3. Identifying and dating a nuclear detonation by decay of mixed fission product activity.

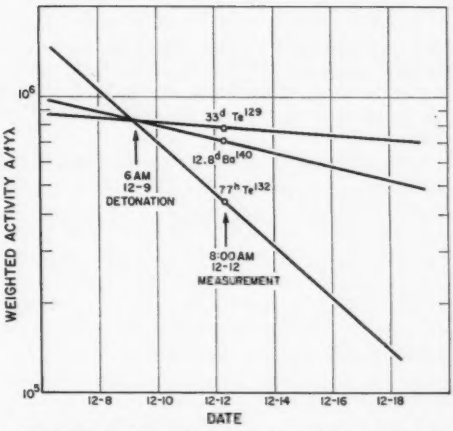

FIGURE 4. Identifying and dating a nuclear detonation by identification of individual fission products.

Rapid Method

These methods have a number of severe limitations:

1. Interference by natural radioactivity or activity from other sources.
2. A non-representative mixture of mixed fission product activity i.e., selective absorption by the atmosphere in the transport from the point of release to the sample collector.
3. The time required to make decay measurements.

These limitations can be vitiated by the determination of the relative concentrations of individual fission product isotopes. The measured activity of a fission product isotope resulting from a nuclear detonation is

$$A = Kfy\lambda e^{-\lambda t} \tag{5}$$

where

A = activity from one fission product isotope
f = efficiency of the detector for the isotope
Y = fission product yield of the isotope
λ = decay constant of the isotope
t = time of measurement after detonation.

Consequently by the determination of the activity of two isotopes

$$A_1 = Kf_1Y_1\lambda_1 e^{-\lambda_1 t} \tag{6}$$

and

$$A_2 = Kf_2Y_2\lambda_2 w^{-\lambda_2 t} \tag{7}$$

whence

$$t = \frac{\ln A_1 f_2 Y_2 \lambda_2 - \ln A_2 f_1 Y_1 \lambda_1}{\lambda_2 - \lambda_1} \tag{8}$$

The time of fission can be determined graphically by plotting $A/fY\lambda$ on semi-log paper. Straight lines are drawn with slopes corresponding to the decay rates of the specific isotopes. The intersection of these lines occurs at the time of fission.

Three isotopes from the air sample from which data was collected for the previous examples were identified by measurement on a gamma-ray spectrometer. The weighted activities were calculated and plotted, as shown in Figure 4, using the characteristic decay rate of each isotope. The single intersection point identifies the activity collected as nuclear detonation debris and the time of fission.

Multiple Detonations

It is meaningless to speak of the birth date or time of fission when a sample consists of mixed fission product activity from more than one detonation. It may be useful, however, to define an apparent age by assuming the observed activity can be characterized as that resulting from a single nuclear detonation. An apparent age can then be calculated from gross decay measurements on the same sample using Equation 4 or by the methods illustrated in Figures 2 or 3. By measurement of the relative activity of individual fission products isotopes, preferably of the same element, an apparent age can be also calculated using Equation 8 or the method illustrated in Figure 4. By any of these methods, the apparent half-life, defined as the length of time required for the activity of a fission product mixture to reach one-half of its value at a particular time, is

$$T_{1/2}(t) = (2^{1/1.2} - 1)t = 0.78t \qquad (9)$$

In general, apparent ages calculated by different methods will not be equal and in the case of calculation from gross decay measurements, will change with time.

An apparent age can also be calculated from three measurements of the gross activity from the same sample by assuming the mixed fission product activity can be characterized by

$$A = t^{-x} \qquad (10)$$

where

t = apparent age
A = activity at time t
x = constant characteristic of the mixture.

The apparent age is then calculated by graphical solution or the method of successive approximation. The apparent half-life would be

$$T_{1/2}(t) = (2^{1/x} - 1) \qquad (11)$$

Solutions obtained by this method are quite variable because of high sensitivity to the particular values of activity used and errors of measurement.

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Biochemical Mechanisms in Chronic Carbon Disulfide Poisoning*

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FOREIGN LITERATURE reports show many instances of poisoning from carbon disulfide (CS_2).[1, 2, 3, 4, 5, 6] A study in the U. S. by the Pennsylvania Department of Labor and Industry, in 1938, reported in detail many injurious effects in workers exposed to CS_2.[7] This investigation was followed by two reports of clinical surveys of workers exposed to CS_2 in 1945 and 1950 by Rubin and coworkers[8, 9] in which they reported psychic and neurologic disturbances from CS_2 exposure.

Since 1950, no clinical reports on the effects of CS_2 exposure in this country have appeared. The authors' (A.E.C., H.J.P.) field experience with CS_2 has demonstrated that the agricultural use of CS_2 in this country still presents a health hazard.[10] A survey of corn bin fumigation operations in 1957[11] revealed concentrations of CS_2 present in the bins in excess of the threshold limit value set by the A.C.G.I.H. of 20 ppm[12] for 5 days following fumigation. These bins were entered by the fumigators, without the protection of an organic vapor canister, three days after fumigation. During the process of fumigation, the rated capacity of 2 per cent organic vapors in air for the safe limit of the vapor canister worn by the sprayers, was exceeded in 14 per cent of the operations sampled at the grain storage sites.

The general purpose of this study has been to determine the relationship that exists between the exposure to the toxic substance and the pathologic lesions which develop: (1) by identifying the chemical reaction of the toxic substance with the body; (2) determining the effect of this reaction product on the body; (3) determining the reaction of the body to this toxic action; and (4) correlating the signs of disabling toxic action with the development of and location of pathologic changes in body tissues.

Masuda[13] had reported paralysis of hind legs in rabbits exposed to 200 ppm CS_2 for six hours per day, six days a week for five months. Sakurai[14] had reported appreciable paralysis of the extremities of rabbits exposed to different concentrations of CS_2 for various periods of time. The exposures varied from 150 ppm CS_2 for four hours per day for 60 days to 300 ppm CS_2 for four hours per day for 100 days. On the basis of these reports, it was anticipated in the present study that paralysis might occur by the end of the third month of exposure at a concentration of 250 ppm CS_2 for six hours per day, five days a week. This did not happen. The concentration was increased to 500 ppm CS_2 at the 17th week and continued for five weeks without signs of paralysis. The concentration was then increased to 750 ppm CS_2 at the 22nd week of exposure and maintained at this level until clinical toxicity became evident, at which time the exposure was terminated.

Experimental

The animals used in this investigation were male New Zealand white rabbits, about six months of age, ranging in weight from 2600 grams to 3700 grams with a mean of 3294 grams. From a total of 17 animals used, eleven were exposed and six were retained as controls. Blood samples (20 ml) were obtained from each rabbit weekly from the central artery of the ear. Two exposed and one control rabbit were sacrificed after 12 weeks of exposure, one exposed and one control rabbit after 28 weeks of exposure, and the remainder of the animals were sacrificed after clinical signs of toxicity occurred. After termination of the exposure, the animals were divided into two groups, four rabbits being sacrificed immediately after exhibiting toxic signs, and four after an observation and recovery period of six to seven weeks. Control animals were sacrificed at the same time with each of the exposed groups. Sacrifice was accomplished by the intravenous injection of 3–4 cc. of Nembutal.

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Exposure Methods and Apparatus

The exposure chamber was a 25 cubic foot chamber constructed of wood. Its plywood sides and back were lined with aluminum foil, and its top was a sheet of aluminum. The opening of the chamber was fitted with a wood and plexiglass door. The floor surface was covered with two coats of black asphaltum, over which a removable metal tray was placed to catch the excrement. Six inches above the floor of the chamber a wire screen was installed to support the animal cages, a separate cage being used for each animal. Inlet and exhaust plenums were installed at the top of the chamber and below the supporting screen. Supply and exhaust air connections to the central air supply and exhaust systems provided the chamber with one complete air change every five minutes. The CS_2 was introduced into the chamber in the vapor state by bubbling nitrogen through liquid CS_2, and the resulting gaseous mixture was metered into the air from the central supply system. Rotameters were connected between the nitrogen and liquid CS_2 supply and also to the intake air stream. The chamber was maintained at a negative pressure of 0.05 inch of water during operation.

Concentration values of CS_2 within the exposure chamber were established by analyzing samples collected through a port in the side of the chamber. The exposure chamber atmosphere was sampled by drawing chamber air through 150-ml gas collecting flasks, which were then injected with diethylamine-copper reagent by means of a hypodermic syringe.[15] The quantity of CS_2 in the sample was determined after 30 minutes by reading the density of yellow color in a Klett colorimeter using the #42 filter. Exposure chamber concentrations were determined four times daily, at the beginning and close of each day and at two hour intervals during the exposure.

Electrophoretic Studies

The electrophoretic determinations were performed on the blood serum from each animal once each week during the experiment, as a screening process for metabolic injury. Both the protein and lipoprotein constituents of serum were measured electrophoretically. These determinations were made in a Durrum-type, Spinco cell and read with a Spinco Analytrol. Sample preparation and staining for protein constituents were performed as described by Jencks, et al.[16] The lipoprotein estimations were made by separating 0.05 ml of serum on a paper strip in a conventional protein run at 5 ma for 16 hours. The strips were dried in a 110°C oven for 30 minutes and stained for three hours with Sudan Black prepared according to Schwan.[17] The strips were then rinsed free of background stain in 50 per cent v/v ethyl alcohol, dried and read on the Analytrol. The results were recorded as scanner units.

The values of 55–60 per cent albumin, 12–15 per cent alpha globulin, 14–16 per cent beta globulin and 14–16 per cent gamma globulin obtained for the control rabbits agree with those reported by Charioni[18] for normal rabbits. It would be difficult to differentiate the control and exposed rabbits based upon the protein values obtained by electrophoresis of the serum. The stability of the serum proteins during this experiment, even though the animals lost weight and developed signs of toxicity, is interpreted as indicating little or no hepatic injury. These observations were substantiated by histologic examination when the animals were sacrificed.

Figure 1 shows that the concentration of beta lipoprotein is 20 to 50 per cent greater in the exposed rabbits than in the control rabbits. The sudden and absolute increase in beta lipoprotein shown in Figure 1, which occurred in both exposed and control groups at the eleventh week, resulted from failure of the air conditioning system in the animal quarters. Due to this failure the animal room and chamber temperatures varied with the daily temperatures during this hot (75–95°F) period in July. Since this incident caused a dramatic change in lipoprotein in both groups, with the larger change taking place in the exposed group, it suggested that the lipoprotein changes found in the exposed rabbits may be a non-specific stress response by the body metabolism.

These examples and the data presented here are interpreted as showing that the exposure of

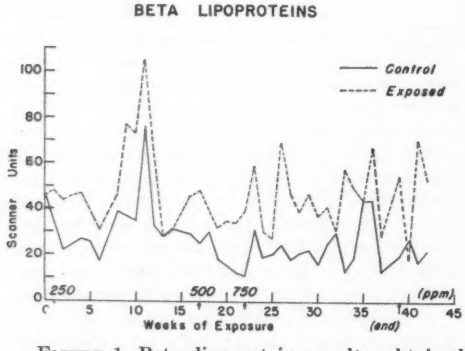


FIGURE 1. Beta lipoprotein results obtained by paper electrophoresis of serum.

the rabbits to CS_2 has produced, in part at least, a stress response in the body. It is further evident from the eleventh week response of the beta lipoprotein that this system responds to the summation of the stress conditions, i.e., to the exposure to CS_2 plus the high temperature of the environment. These observations indicating CS_2 exposure as a condition of stress in the animal were substantiated by the enlarged adrenals (Table I) found grossly and histologically in the exposed animals at sacrifice.

TABLE I
Wet Weight of One Adrenal Gland

Time of sacrifice after exposure	Adrenal weight	
	Mean	Range
<2 weeks	0.48 g	0.31–0.72
>4 weeks	0.43 g	0.22–0.57
Control	0.29 g	0.15–0.41

Ultraviolet Absorption Spectra of Serum

In the present study, serum samples taken from the rabbits before exposure were stored in stoppered test tubes in the refrigerator at 4°C. At intervals, after the start of the exposures, the ultraviolet absorption spectra of the sera from individual animals were compared with the pre-exposure sample in a Beckman DK-2 spectrophotometer. The comparison was made as follows:

> Serum samples were diluted with saline to provide 5 per cent solutions of sera. The diluted sample taken prior to exposure was placed in the reference cell. The diluted serum sample obtained from the same animal after the start of exposure was then placed in the sample cell and read against the pre-exposure sample over the wavelength range of 240–360 mμ.

An example of the results obtained by this method is given in Figure 2.

The curve 9-11-57 of Figure 2, from a serum sample obtained from a rabbit two weeks after increasing the exposure concentration to 500 ppm CS_2, contains the 250 and 292 mμ transmittance minima, thus providing evidence that the thiocarbamate is present in the blood serum of the rabbit. The curves obtained with sera from the same animal on 7-15-57, 8-21-57, and 9-25-57 show the 280 mμ transmittance minimum which is evidence that the 2-thio-5-thiazolidone concentration in the blood serum of the animal increases with exposure time. This evidence shows that some of the CS_2 inhaled by the animal during the daily exposures combined with the proteins in the rabbit serum and tended to become cumulative especially after the exposure was increased to 500 ppm CS_2.

The large increase in transmittance shown in the 8-21-57 and 9-25-57 curves between 250 mμ and 290 mμ are suggestive of structural alterations taking place in the animal serum composition. This phenomenon was observed in all of the exposed animals and was also found occasionally in the control animals. The only interpretation

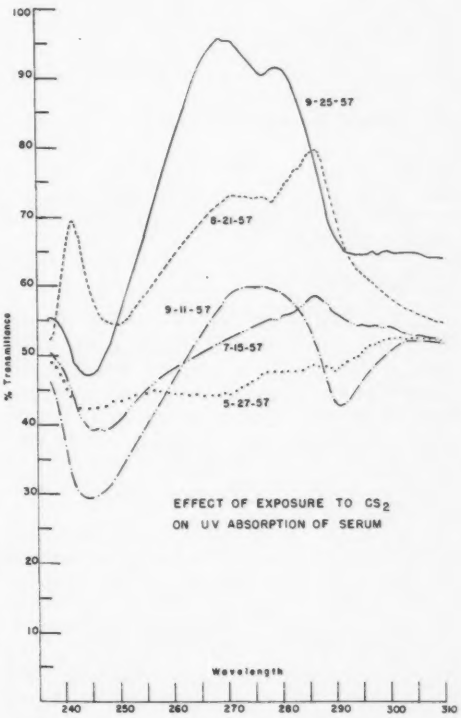


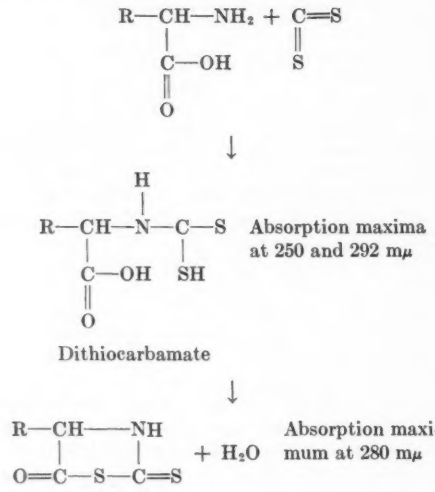
FIGURE 2. An example of the change in ultraviolet absorption spectrum of 5 per cent serum read against a pre-exposure sample of serum.

placed upon this shift in transmittance is that exposure to CS_2 accelerates and intensifies a process which is normally taking place in the aging rabbit. Further work on the significance of this shift is needed.

Chelation of Metal Ions

The chemical reaction of CS_2 with free amino groups of proteins or amino acids may be pre-

sented as follows:



As described in the previous section, the development of absorption bands characteristic for both of these products has been found in the ultraviolet spectra of sera from the exposed rabbits. These substances are known to form non-ionized complexes with polyvalent metal ions. According to Martell and Calvin[19] the order of stability of the chelates of certain biologically important ions should be Cu > Co > Zn > Fe > Mn > Mg. As the concentrations of the dithiocarbamate and thiazolidone groups increase in the animal body, the amount of polyvalent inorganic elements bound to protein derivatives in the cells of the exposed tissues should increase. Accordingly, ionic concentrations of these elements must decrease within the body cells when the rate of formation of metal binding groups exceeds the rate at which the ions are supplied to the cell. Therefore, the lesions produced under these conditions should simulate the trace metal lesions described in nutritional studies.

In the heterogeneous ionic environment found in a living cell, the total amount of any given inorganic element which will be present within the cell will be proportional to the product of Ke $(IS_1 + IS_2 + IS_3 \cdots)$ where Ke is the concentration of the chelating agent and IS is the product of the concentration of the inorganic element and its chelate stability constant. Therefore, even though the magnesium chelate is the least stable, the element could still be present in the cell as a chelate in appreciable quantities because the molar concentration of magnesium in the cell is greater than that of other polyvalent ions which form chelates.

This formation of chelating structures by CS_2 in the body and their sequestering action on the polyvalent ions necessary for normal cell metabolism presents a basic mechanism for understanding the slow and insidious nature of CS_2 toxicity and its pathologic effect on the body tissues. It is believed that this concept, which represents a previously undescribed type of toxicity, is supported effectively by the results obtained in this investigation.

Serum and Tissue Alkaline Phosphatase Activity[a]

Chelating agents have been shown to inhibit the activity of this enzyme by sequestering the activating ions.[20, 21] The identification of dithiocarbamate and thiazolidone groups in the serum of the exposed animals and the observed increase in the urinary excretion of zinc, reported below, suggested that the chelation of metal ions by the CS_2-protein reaction products could affect enzyme systems requiring metal ions as activators. Alkaline phosphatase is an enzyme system of this type in which zinc and magnesium ions, under suitable conditions, have been shown to be activators.[21]

Preliminary tests with added zinc were carried out on sera of control and exposed animals. Data obtained from these tests, supported by reports in the literature[22, 23] indicated that further studies on the addition of zinc to the enzyme test system would not be advantageous. Accordingly, attention was directed to the influence of added magnesium. First observations indicated markedly different degrees of activation between exposed and control sera. The procedure outlined below was employed throughout the investigation.

Alkaline phosphatase activity was determined by Seligman's method.[24] The only changes made in the procedure were the addition of one ml of water to the test system when no magnesium was added (measurement of "basic activity") and replacement of this by one ml of 0.01*M* magnesium solution when measuring activity in the presence of magnesium. The substrate used in this procedure is beta-naphthol phosphate.[b] Activity was determined by measurement of liberated beta-naphthol, but is expressed in terms of mg of phosphorus equivalent to naphthol split from substrate in one hour at 37.5°C per 100 ml of serum or one gram of tissue.

The data in Table II show that basic activity

[a] Preliminary report was presented by John T. Mountain and Fulton R. Stockell, Jr., at the 132nd meeting of the American Chemical Society, New York, September 1957.

[b] Available from Dajac Laboratories, The Borden Co., 5000 Langdon St., Philadelphia 24, Pa.

TABLE II

Effect of CS_2 Inhalation on Serum Alkaline Phosphatase Activity

The values of week #40 were obtained two weeks after the termination of exposure. Activity = mg P/hr/100 ml serum.

Week	Basic activity		Activity with added Mg^{++}	
	Control	Exposed	Control	Exposed
8. Mean	3.0	2.0	3.7	3.9
Range	2.9–3.2	1.5–2.4	3.4–3.9	2.6–5.1
12. Mean	2.3	2.2	2.6	3.0
Range	1.3–2.6	1.6–3.4	1.8–2.9	2.0–4.0
16. Mean	1.8	1.6	2.1	2.4
Range	1.4–2.1	1.3–2.3	1.7–2.4	2.0–3.6
20. Mean	1.7	1.3	1.9	2.0
Range	1.5–1.9	1.0–1.5	1.5–2.3	1.7–2.7
24. Mean	1.6	1.3	2.1	2.2
Range	1.2–1.7	0.8–1.9	1.6–2.3	1.4–3.3
28. Mean	1.4	1.3	1.8	2.1
Range	1.0–1.9	0.9–1.8	1.1–2.3	1.3–3.2
32. Mean	1.3	1.2	1.7	1.6
Range	0.9–1.7	0.8–1.8	1.2–2.4	1.0–2.6
36. Mean	1.4	1.3	1.9	1.8
Range	0.8–2.2	0.6–2.3	1.1–2.3	0.9–3.5
40. Mean	1.0	1.3	1.9	1.6
Range	0.5–1.4	0.4–1.3	0.9–3.4	1.0–2.0

of the serum alkaline phosphatase of the exposed animals was 10–30 per cent lower than in the serum from the controls. Upon the addition of magnesium to the test system the enzyme activity of the exposed rabbit serum was increased 30 to 70 per cent while the control activity only increased 5–40 per cent. From the eighth through the 31st week the average activity, with magnesium added, for the exposed animals exceeded that of the control group. These data suggest that the concentration of activating ions in the serum of the exposed animals was low enough to limit the alkaline phosphatase activity of the serum.

At the time of sacrifice, tissue specimens of cerebrum, kidney, liver and lung were removed for tissue alkaline phosphatase activity measurements. The tissue specimens were weighed, ground in a Latapie grinder and homogenized with a Potter-Elvehjem pestle. The general preparation of tissue macerate for enzymatic activity assay followed the procedure of Reis.[25] After autolysis for three days at 4°C, part of the macerate was subjected to dialysis for two hours in an Aminco stirring dialyzer against four changes of cold distilled water. Dialyzed and nondialyzed aliquots were diluted so that one ml of tissue suspension contained the equivalent of one mg of original tissue. The determinations of alkaline phosphatase activity were then carried out as for serum.

The changes in observed alkaline phosphatase activity due to incubation in the absence of, and in the presence of, 0.0014*M* magnesium ions are presented in Table III.

It is clearly shown that without the addition of magnesium ions the activity, as determined, is limited to about half of the activity obtained in the presence of added magnesium ions. This was true whether the tissue was dialyzed or not. Therefore, by studying only the activity resulting from the addition of a known excess of Mg^{++} ions it is possible to evaluate the relationship of this activator to the total enzyme concentration.

In section C of Table III it is shown that the activation by magnesium of the enzyme in the

TABLE III

The Effect of CS_2 Exposure on the Tissue Alkaline Phosphatase Activity

Tissue	Controls (av. of 4)		Exposed (av. of 9)	
	Crude	Dialyzed	Crude	Dialyzed
A. Activity* in the presence of .001*M* Mg^{++}				
Cerebrum	1.49	2.06	2.28	1.96
Kidney	17.66	17.38	17.17	18.15
Liver	4.02	4.14	4.01	3.69
Lung	3.20	2.51	5.95	5.68
B. Activity in the absence of added Mg^{++}				
Cerebrum	0.91	1.51	1.36	1.02
Kidney	12.05	10.81	10.15	8.59
Liver	1.85	2.01	2.09	1.50
Lung	1.12	1.02	3.38	2.30
C. Increase in activity due to addition of Mg^{++} (A–B)				
Cerebrum	0.58	0.55	0.92	0.94
Kidney	5.61	6.57	7.02	9.56
Liver	2.17	2.13	1.92	2.19
Lung	2.08	1.49	2.57	3.38
D. Ratio of increases in activity: Dialyzed/Crude				
Cerebrum	0.95		1.02	
Kidney	1.17		1.36	
Liver	0.98		1.14	
Lung	0.72		1.32	
E. Percent increase in Mg^{++} sensitive activity in tissues from the exposed animals compared to controls				
Cerebrum	7%			
Kidney	19%			
Liver	16%			
Lung	60%			

* Activity = mg P/hr/gm of tissue.

tissues from the exposed animals, with the exception of the liver, is 30–40 per cent greater than in the unexposed control animals. This is most clearly demonstrated by the dialyzed tissues where activators and inhibitors, other than magnesium, normally present in the tissues have been removed.

To eliminate dialysis as a factor and to demonstrate the above observation, the results in section D of Table III are presented as ratios of activity in the dialyzed samples to original activities. Thus, if an activator were removed by dialysis the measured activity in the dialyzed sample would be reduced and the ratio obtained would be less than one as is shown for lung tissue in the control group. However, if no activator or inhibitor of the enzyme is removed by dialysis then there should be no change in the total activity and consequently the ratio should be equal to one as is shown for the cerebrum and liver in the control group and the cerebrum of the exposed group. If then, an inhibitor of the enzymatic activity is removed by dialysis the total activity measured in the dialyzed sample should increase and the ratio should become larger than one as shown for the kidney of both groups and the liver and lung of the exposed group. Thus, in the case of the kidney, liver and lung of the exposed group, the presence of a significant amount of alkaline phosphatase inhibitor is indicated. This inhibition, based on the experimental evidence, can be best interpreted as due to the presence and chelating effect of thiocarbamate and thiazolidone groups on dialyzable amino acids in the tissues of the exposed animals.

In the discussion of Table III above, it has been pointed out that the tissues of the exposed animals contained both a larger amount of alkaline phosphatase and an inhibitor of this enzymatic activity. Since the ratios calculated for the exposed and control tissues in section D are expressed in terms of units of final activity per unit of original activity in the presence of excess magnesium ions, it is possible to estimate the percentage increase in enzyme concentration in the tissues from the exposed animals. These results are given in section E of Table III.

It is noteworthy that in the lung, the tissue directly exposed to CS_2, 60 per cent more enzyme activity was found than in the unexposed control. In the tissues more remote from direct exposure, the liver and kidney, smaller increases in the magnesium sensitive activity were found. The figure given for the cerebrum is of doubtful significance and is only included for completeness of data.

These findings are consistent with the proposed chelating effect of the CS_2-protein derivatives on the inorganic ion concentration of the body fluids.

Changes in Zinc Metabolism

In a previous publication[10] on the effects of cutaneous exposures of rabbits to CS_2 vapor, data were presented which demonstrated the marked depletion of zinc levels in the blood serum and cells following single six hour exposures to 130 or 750 ppm concentrations of CS_2 in air. Because of the intermediate position of zinc in the order of chelate stability constants and its importance in nutrition as a trace element, it was selected for careful study in this experiment. Urine, feces, blood sera, red blood cells and body tissues were analyzed for zinc as a representative inorganic ion which would form chelates with dithiocarbamate and thiazolidone groups if they were formed in the tissues of the animal body.

Due to a limited supply of metabolism cages, it was possible to perform the excretion studies on only the exposed animals. The metabolism cages were constructed of stainless steel and provided with stainless steel wire screen inserts to separate the feces from the urine. The urine of each exposed rabbit was routinely collected over the weekend and in certain cases on a daily basis. Borosilicate glass beakers, cleaned with dichromate cleaning solution and thoroughly rinsed with distilled water, were used for urine collections. The precipitation which normally takes place in rabbit urine was prevented by adding 10 ml of 1:1 redistilled HCl to each beaker prior to the urine collection. Samples (2.5 gm) of the daily or weekend fecal specimens were weighed into chemically clean Phillips beakers.

Blood samples (20 ml) were withdrawn from the central artery of the rabbit ear in chemically clean syringes once a week and placed in chemically clean test tubes to clot. After clot retraction and centrifugation, the serum was separated from the cells and each portion stored in clean test tubes until analyzed.

When the animals were sacrificed, specimens of 11 body tissues, including bone, were obtained for zinc analysis. The brain and spinal cord specimens were also analyzed for copper for the reasons set forth in the subsection on the copper content of these tissues.

All samples were wet ashed with redistilled nitric acid. The residues were evaporated to dryness twice with redistilled HCl, dissolved in one ml of HCl and diluted to volume with distilled water. Zinc was then determined by a mixed color dithizone method on suitable aliquots of the sample solution.[26]

Zinc Excretion

During the first week of exposure to 250 ppm CS_2, there was a three-fold increase in the mean

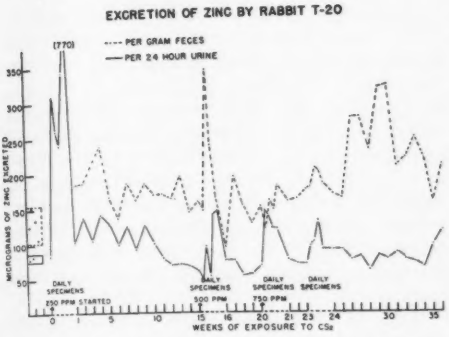


FIGURE 3. Excretion of zinc by a typical rabbit, T-20.

concentration of zinc in the daily urine samples. Daily urinary zinc concentrations for rabbit T-20 (Figure 3), during the first and other designated weeks of exposure at different periods of the study, exhibit marked increases in the urinary zinc excretion. The more normal urinary zinc values obtained in the samples collected on the weekends following five-day exposure periods, are consistent with the known short term effect of chelating agents on the excretion of toxic substances. The results shown for one rabbit (T-20) on a daily collection basis in Figure 3 are illustrative of the type of response obtained in the urinary zinc excretion for the exposed group. These results are interpreted as indicating that the complexing action of dithiocarbamate on body zinc has increased the elimination of this element only during the five day exposure period. It has been reported that a similar renal loss of zinc occurred during EDTA administration.[27]

The fecal zinc excretion is presented as micrograms of zinc per gram of feces in Figure 3 for rabbit T-20. As shown in the daily excretion data for rabbit T-20, the feces contained a higher concentration of zinc during five-day periods of exposure than was found in the weekend samples. These rapid changes in level of excretion in both the urine and feces coincident with the period of CS_2 exposure provide additional evidence that the chelating activity of the CS_2 reaction products in the body have a depleting effect on the inorganic ions available to the body tissues.

Following the increase in the exposure concentration to 750 ppm of CS_2, the fecal zinc excretion increased for the exposed group. This increase demonstrates that the level of zinc excretion is proportional to the CS_2 concentration inhaled and substantiates the interpretation that the chelating activity of the dithiocarbamate and thiazolidone, resulting from the reaction of CS_2 with free amino groups in the body, can alter the inorganic metabolism of the body. The continuous increase in zinc excretion in the feces, observed during the period of weight loss starting with the 24th week, may also be related to the tissue breakdown taking place during this toxic stage of the study. This result was obtained even though the food consumption and consequently the quantity of ingested zinc decreased during this time.

Serum Zinc

The minimal, maximal, and mean serum zinc values of nine exposed and four control rabbits are presented in Figure 4 for the pre-exposure period, and for each four-week exposure period.

Examination of the data provided in this figure reveals the following points: (1) the minimal values for the exposed group are consistently lower than those of the control animals; (2) there is a steady drop in the maximal values of the exposed group throughout the exposure period while the control group's maximal zinc values tend to rise; and (3) the mean serum zinc levels of the exposed animals, while higher than those of the control group prior to exposure and during the first nine weeks of the study, then dropped below the mean zinc levels of the control group and remained there for the duration of the exposure period. It is evident that these observed changes are not striking and individual values may fail to indicate an animal as being either an exposed or a control. They do, however, indicate a gradual depletion of body zinc during the experiment. By the nature of the experiment, no information concerning the state of the zinc, ionic

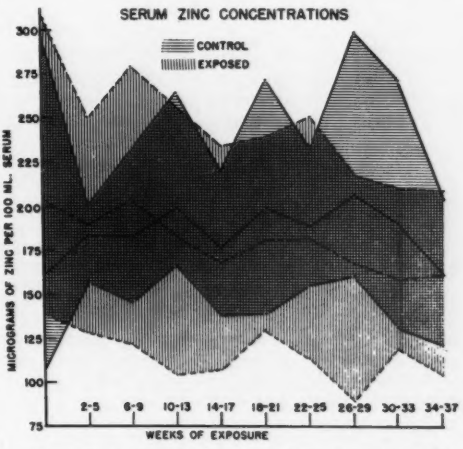


FIGURE 4. Total zinc concentration in rabbit serum from control and exposed groups.

or bound, in the serum is known. However, the increased excretion of zinc reported above and the gradual fall in serum zinc levels would indicate that much of the serum zinc may be in the bound form. The values obtained by determining the total zinc in the red blood cells showed a similar variation in the exposed animals.

Effect on Inorganic Element Levels in Tissues

In presenting the interpretation of the effect of CS_2 exposure on the tissue content of zinc and copper the following basic assumptions are made:

(1) There are present in the protein structures of the body an infinitely large number of free amino groups with which the CS_2 can react to form thiocarbamate and thiazolidone groups.

(2) That the metabolism of the cells in any tissue may be disturbed, but not sufficiently to cause death and loss of cell contents.

(3) For purposes of calculation, it is assumed that all of the inorganic element found in exposed animal tissues in excess of the amount found in the tissues of the control animals is bound by chelation with thiocarbamate or thiazolidone groups to tissue components.

These assumptions are supported by the observations that CS_2 was found in the exhaled breath 16 hours after exposure was terminated, and by the appearance in the serum of exposed animals of ultraviolet absorption changes which have the characteristics of known protein thiazolidone absorption. It was also shown that the amount of absorption by the exposed sera increased as the exposure time and concentration increased indicating that the CS_2 was accumulating in the blood sera as the thiazolidone.

At the time of sacrifice, specimens of 11 body tissues were obtained from each exposed and control animal and analyzed for total zinc content. Brain and spinal cord samples were also analyzed for total copper content. The results of the zinc analyses are presented in Table IV as individual and mean values for each of three groups of rabbits. The first group of animals was sacrificed within two weeks after the last exposure, the second group represents animals sacrificed four to six weeks after exposure and the last group represents the unexposed control animals.

Table IV shows that, in the group of animals sacrificed within two weeks after the last exposure, nine of the eleven tissues analyzed contained more zinc than the control animal tissues. That this excess of zinc found in the tissues is related to the CS_2 exposure is shown by the group of animals sacrificed four to six weeks after exposure, where six of the 11 tissues analyzed showed total zinc content to be returning toward the control values. This observation becomes substantially important since the bulk body tissues of bone, muscle, liver and kidney all showed this recovery to a marked degree. This evidence substantiates the hypothesis that CS_2 reacts with the proteins of the body to form binding sites for polyvalent ions circulating in the body fluids. These data are also considered further evidence that the toxic reaction product of CS_2 with body tissues can interfere with the metabolic function of inorganic ions in the body.

A concise summary of the changes in tissue zinc levels in the two groups of exposed rabbits is presented in Table V.

TABLE IV

Analysis of Body Tissues for Zinc and Copper (mM/Kg) and their Correlation with Pathologic Changes

	mM Zn/Kg			mM chelated Zn/Kg		mM Cu/ Kg			Pathology
	1	2	3	1	2	1	2	3	
Bone	2.52	2.35	2.00	0.52	0.35				Normal
Muscle	0.30	0.21	0.15	0.15	0.06				Normal
Lung	0.29	0.35	0.26	0.03	0.09				Normal
Pancreas	0.67	0.46	0.45	0.22	0.01				Normal
Spleen	0.38	0.34	0.30	0.08	0.04				Hyperactive follicles
Adrenal	0.30	0.36	0.36	−0.06	0.00				Enlarged (tumor)
Liver	0.60	0.50	0.52	0.08	−0.02				Cellular fatty degeneration
Kidney	0.50	0.44	0.40	0.10	0.04				Glomerular nephrosis
Cerebrum	0.22	0.24	0.22	0.00	0.02	0.13	0.15	0.24	Cellular degeneration
Cerebellum	0.18	0.18	0.20	−0.02	−0.02	0.19	0.15	0.16	Perkinje cell degeneration
Spinal cord	0.22	0.17	0.18	0.04	−0.01	0.33	0.89	0.90	Pyramidal tract degeneration

Column 1 = Average of 7 animals sacrificed <2 weeks after last exposure.
Column 2 = Average of 4 animals sacrificed >4 weeks after last exposure.
Column 3 = Average of 5 unexposed controls.

The percentage increases in the mean zinc concentrations in the eleven body tissues are given for the group sacrificed within two weeks and for the group sacrificed four to six weeks after the final exposure. This table clearly shows the degree of recovery toward normal tissue zinc levels during the four to six week recovery period.

As shown in Table IV, the bulk body tissues muscle and bone contained more zinc than was found in the control animals. Therefore, using the above assumptions, the minimum concentration of chelating groups present in the body tissues of the exposed animals must be stoichiometrically equivalent to the analytically determined excess quantity of zinc bound in any tissue. Since the sequestering action of the chelating agent is not specific for any single element, it is emphasized that the calculated concentrations of chelating agent based upon the zinc analysis represents a quantitative estimate of only the minimum concentration of chelating agent present in the tissues. However, the change in concentration of chelating agent, which causes profound change in the metabolically available inorganic ions in the tissue cell, provides the basis of this type of toxicity. An illustrative calculation of this kind is given below.

Schematically, the following reactions may illustrate the mechanism of this type of toxicity:

$$CS_2 + RNH_2 \leftrightarrows RNHC(=S)SH$$

Thiocarbamate
Chelating agent (Ke)

$$2Ke^- + Zn^{++} \leftrightarrows ZnKe_2$$

Using the bone zinc values as representative of the inorganic metabolism disturbance in the animal due to exposure to a chelate producing agent, the following calculation of the minimum chelate concentration is presented:

165 mg of Zn/kg − 131 mg Zn/kg

= 34 mg excess Zn/kg of bone.

Or expressed as millimoles (mM)

2.52 mM Zn/kg − 2.00 mM/kg

= 0.52 mM excess Zn/kg of bone.

As two mM of chelating agent are required to complex one mM of zinc the minimum concentration of chelating agent is then 1.04 mM/kg of bone.

Using these calculated values and a dissociation constant for the zinc chelate of 1×10^{-10} moles/liter[19] or 1×10^{-4} mM/liter (or kilogram) the maximum concentration of zinc (Zn^{++}) ion in equilibrium with the estimated quantity of chelated zinc is calculated as follows:

$$\frac{(Ke)^2(Zn^{++})}{(ZnKe^2)} = 1 \times 10^{-4} \text{ mM/kg}$$

$$x = (Zn^{++})$$

$$2x = (Ke^-)$$

then:

$$\frac{(2x)^2(x)}{(0.52)} = 1 \times 10^{-4}$$

$$4x^3 = 5.2 \times 10^{-5}$$

$x = 2.35 \times 10^{-2}$ mM Zn^{++}/kg of bone.

When compared with the mean initial concentration of zinc found in the bone of control rabbits, i.e., 2.00 mM/kg, a concentration known to be metabolically available, it becomes apparent that the presence of chelating structures in the body tissues has limited the ionic concentration of zinc in the bone tissue to approximately 1 per cent of the initial total.

Since much of the evidence accumulated in this study has shown that CS_2 exposure interferes with inorganic ion metabolism in the animal body, it was considered desirable to analyze the brain and spinal cord for copper. This element is normally present in higher concentration than zinc in these rabbit tissues and its metabolic function as a structural part of cytochrome oxidase and coenzyme A dehydrogenase may more directly affect the life of the nerve cells if copper becomes unavailable for normal metabolic functions. Furthermore, copper is

TABLE V

Per cent Increase of Total Zinc Levels in Tissues of Rabbits Exposed to Carbon Disulfide

Tissue	Rabbits sacrificed within two weeks of final exposure %	Rabbits sacrified 4–6 weeks after final exposure %
Bone	26.0	17.6
Muscle	94.0	36.0
Lung	8.7	33.7
Pancreas	50.2	2.4
Spleen	27.2	13.8
Adrenal	−17.0	−0.9
Liver	14.6	−0.5
Kidney	26.5	10.4
Cerebrum	3.5	8.5
Cerebellum	−9.8	−11.3
Spinal cord	21.0	−9.2

known to be bound more tightly than zinc by thiocarbamate or thiazolidone groups. The results of this study are given in Table IV.

As mentioned above, the exposed animals were sacrificed in two groups, seven of the exposed animals were sacrificed within two weeks after exposure was terminated while the remaining four exposed animals were sacrificed four to six weeks after the exposure was terminated. The data in Table IV show that in the animals with severe pathologic changes, sacrificed within two weeks after the termination of exposure, the copper content of the spinal cord and the cerebral cortex was found to be only half that found in the controls. The copper content of the cerebellum of these animals was found to be a little above that found in the control animals.

In the animals sacrificed after the observation period of four to six weeks, the copper content of the spinal cord had returned to normal levels, but the severity of the pathologic lesion was not changed as estimated from histologic examination. However, in the cerebellum, the pathologic lesions appeared to be decidedly improved and the copper content of these tissues was found to be only slightly below the values found in the control rabbits. In the cortex an increase in the copper content of the tissues was accompanied by an improvement in the pathologic changes.

In one control animal (T-24) which sustained a dislocated vertebra during the course of the experiment, the copper content of the injured tissue was found to be reduced to less than half the normal level during the week following the injury.

In rabbit T-7, of the exposed group, an acute esophagitis developed during the 29th week of exposure and he was sacrificed. No pathologic change was observed in the spinal cord and only minimal changes were found in the brain. The copper content of the spinal cord was found to be 55 per cent higher than the mean normal value while that of the brain tissue was found to be normal.

It would appear from the data given in Table IV and the observations reported on T-7 and T-24 that the exposure to CS_2 results in the formation of metal complexing groups in the brain and spinal cord tissue. The presence of these complexing groups increases the amount of copper bound in the tissue if no pathologic change involving cellular degeneration is found. However, after cellular degeneration has progressed to become visible pathologically there is also a loss of copper from the injured tissue (Table IV spinal cord and cortex). That the pathologic changes and the copper content of the central nervous system are the result of the CS_2 exposure is shown by the improvement of both of these factors after the exposure was terminated.

These observations suggest that the interference by thiazolidone and thiocarbamate with proper metabolic function of copper in the spinal cord may produce the lesions observed. This suggestion is strengthened by the failure to obtain evidence for fat which is normally found in the axon cylinder in the spinal cord. Interference with the known[28] function of copper as an essential component of the coenzyme A dehydrogenase system is compatible with the above observations and with the type of lesion found in the spinal cord.

Although the relationship of the copper content of the tissues to the pathologic lesions observed is not completely demonstrated by the data given in Table IV, enough data are present to indicate that a cause and effect relationship may be found between the state of the copper in the nervous tissue and the state of development of the pathologic lesions when deliberate experiments are performed to test this. Thus, the chelating activity of CS_2 when combined with tissue proteins may provide a tool for studying the metabolic function of copper in brain and spinal cord tissue, and the type of lesions produced by removing this element from the metabolic cycle of these tissues.

Serum Cholesterol Changes

The serum of all rabbits was analyzed each week for free and total cholesterol by Zak's[29] method. The results of these analyses were averaged for each four week period and are shown in Table VI. During the first five months of exposure to 250 and 500 ppm of CS_2 for six hours a day, five days a week, no significant change in total or esterified cholesterol was found. However, after the animals began to lose weight, while being exposed to 750 ppm, an elevation in the total serum cholesterol was found. That this change is associated with the loss of weight is shown by the prompt return to normal serum cholesterol levels after exposure was terminated and the animals began to gain weight.

Recent reports in the literature[30] indicate that the level and kind of protein in the diet of animals has a decided influence on the metabolic control of cholesterol found in the serum. Since the diet consumed by the rabbits in this experiment contained 15 per cent protein, it is suggested that the failure of our results to substantiate the previously reported[14, 31] changes of cholesterol following CS_2 exposure may be caused by dietary differences. These observations of a protein effect on cholesterol metabolism and

TABLE VI

Cholesterol and Cholesterol Esters Concentrations

The top horizontal line represents pre-exposed values; during months 1–4 the concentration was 250 ppm CS_2 ; during month 5 the concentration was 500 ppm CS_2 ; during months 6–9 the concentration was 750 ppm CS_2 ; the bottom line represents values obtained after the termination of exposure.

Month	Total cholesterol (mgm/100 ml serum)		Cholesterol esters (per cent)	
	Control	Exposed	Control	Exposed
0. Mean	42	42	66	70
Range	36–56	32–55	62–72	59–78
1. Mean	39	41	—	—
Range	34–43	34–59	—	—
2. Mean	43	44	79	78
Range	32–49	31–59	71–84	73–80
3. Mean	36	40	66	67
Range	33–37	30–48	62–69	62–72
4. Mean	34	37	71	68
Range	29–40	31–47	67–72	62–78
5. Mean	37	39	67	61
Range	34–48	32–57	58–75	46–73
6. Mean	39	52	73	72
Range	34–49	42–64	67–84	63–78
7. Mean	38	55	69	63
Range	30–43	38–80	62–77	56–73
8. Mean	40	51	72	68
Range	32–56	45–69	64–80	59–73
9. Mean	33	50	75	68
Range	28–45	41–74	41–74	63–73
10. Mean	36	40	66	60
Range	28–53	25–54	60–71	57–64

weight loss, coincident with a rise in cholesterol of the serum, indicate that the cholesterol changes observed are not a direct toxic manifestation of CS_2 but are probably an indirect result of altered body metabolism. The cholesterol changes may result from the mobilization of body fat from depots and its utilization during a state of weight loss and negative nitrogen balance.

Body Weight Changes

The average weight of the exposed group of rabbits prior to exposure was 3285 grams and that of the control group was 3305 grams. Body weights on all animals in the experiment were recorded at the end of each week. The data obtained for the exposed and control groups are shown in Figure 5.

During the first 11 weeks of exposure, at 250 ppm, the exposed animals failed to gain body weight, while during the same period the controls gained an average of 400 grams in body weight. These data, the changes found in serum alkaline phosphatase activity, and the abnormal beta lipoprotein discused above are evidence that toxic effects were present at this level of exposure. From the 11th week to the 24th week the exposed animals showed a steady gain in weight which was slightly slower than that of the control animals, even though the exposure concentration from the 17th week to the 22nd week was increased to 500 ppm of CS_2 . This shows that the animals were able to accommodate to the metabolic stress caused by the CS_2 inhalation at 250 and 500 ppm levels of exposure.

Two weeks after the exposure was increased to a concentration of 750 ppm CS_2 the exposed rabbits began to lose weight and continued to lose weight until the 37th week. During this period the animals developed obvious physical signs of toxicity and were removed from the exposure chamber as these signs were observed. All exposures were terminated at the end of the 38th week. Following this, the rabbits began to regain some of their body weight, during the four to six weeks observation period prior to sacrifice, indicating that CS_2 toxicity was definitely the cause of the loss in body weight.

In the early part of the experiment, when it was observed that the exposed rabbits were not gaining weight, the food intake was measured to obtain data which might explain the failure to gain. These data are given in Figure 6. The data show that the food intake for the exposed group remained constant up to the 24th week when the loss in weight began. Food consumption then dropped slightly until the 37th week indicating that the toxic effect also reduced the animals'

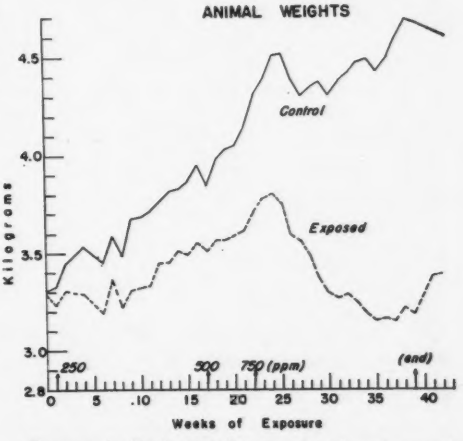


FIGURE 5. Body weight of exposed and control animals.

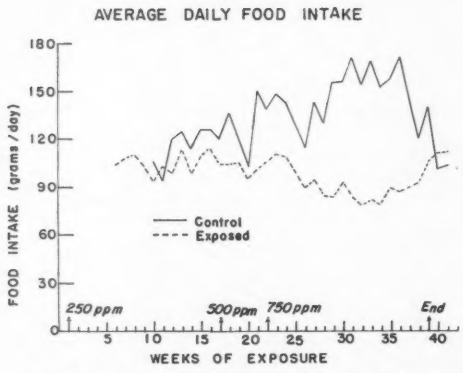


FIGURE 6. Average daily food intake of exposed and control animals.

appetites. Here also, the loss of appetite is shown to be part of a toxic manifestation of CS_2 since during the four week observation period following termination of exposure an increase in food consumption occurred.

The failure of food consumption to increase proportional to the body weight gain during the 11th to 24th week may be the chief reason the body weight gain of the exposed animals was slightly slower than the controls. That the loss in body weight after the 24th week is due to CS_2 toxicity is substantiated by the observation that the food consumption was increasing from the 32nd to the 38th week while the rabbits continued to lose weight. It is apparent from these observations that the animal body can accomodate to concentrations of 250 and 500 ppm of CS_2 on this diet but is unable to adjust to 750 ppm of the vapor. Thus, the threshold concentration of CS_2 which is toxic when inhaled by rabbits on a good diet exposed six hours a day for five days a week is between 500 and 750 ppm.

In this experiment the onset of weight loss preceded the obvious loss of muscular control by six to fourteen weeks.

Respiratory Function of Rabbits Exposed to CS_2 Inhalation

During the course of the experiment no respiratory difficulties were observed during or after the daily exposure. As a routine procedure the respiratory function of each animal was measured before it was sacrificed. The closed circuit respirometry was carried out as reported in a paper by Scheel, et al.[32] In addition, the intrapleural pressure changes were measured by placing an 18 gauge needle connected to a rubber diaphragm through the chest wall at the second interspace above the xiphoid process to the right of the sternum. The pen writer arm attached to the rubber diaphragm traced the movement of the diaphragm on graph paper mounted on a kymograph drum. The results obtained by these techniques are given in Table VII.

The data given in Table VII show that the repeated exposures to CS_2 have not affected the primary function of the lung as indicated by the oxygen consumption, minute ventilation and respiratory rate. However, the expiratory pressures and expiratory time values indicate that the exposed animals have definite mechanical limitations on their breathing capacity. In the data it is shown that most of the control animals (one exception) display a positive pressure (+2.3) phase during expiration when the needle is in the intrapleural space, indicating that under these experimental conditions a forced expiration was taking place. In contrast, the exposed animals showed only a minimal or no positive pressure phase of breathing indicating that no forced expiration phase was present. This observation is substantiated by the increased expiration time seen in the exposed groups of animals. Thus, the expiration phase of the respiratory movement is indicated as being completely passive in the exposed group.

At the time these measurements were made all of the exposed animals had signs of CS_2 toxicity which involved the loss of muscular control over the voluntary movement of the rear legs. It is suggested that, although the sign of loss of muscular control was not visible in the area of the thoracic cage and abdomen, a loss of intercostal and abdominal muscular control would cause the changes observed in the intrapleural pressures. This interpretation is compatible with the location of the pathologic changes found in the

TABLE VII

	Exposed		Control	
	7 rabbits		5 rabbits	
	Mean	Range	Mean	Range
Respirations/minute	63	45–85	53	45–70
Minute ventilation	2440	1480–3600	1644	1331–2080
Oxygen consumption	113	78–148	115	74–159
Inspiration time	0.36	0.20–0.50	0.40	0.20–0.60
Expiration time	0.62	0.40–1.00	0.50	0.20–0.70
Inspiration pressure	−3.96	−3.40–−4.90	−4.23	−4.00–−5.90
Expiration pressure	−0.46	−0.95–+0.50	+2.30	−0.80–+3.00

Minute ventilation and oxygen consumption = cc/minute.
Inspiration and expiration time = seconds.
Inspiration and expiration pressure = inches of water.

spinal cord which are described in detail later in this report.

CS_2 in the Exhaled Breath

During the studies of skin absorption of CS_2 vapors[10] it was observed that the repeated exposure of rabbits to 1500 ppm of CS_2 might be cumulative since the CS_2 in the exhaled breath increased on successive exposures. To check this the CS_2 in the exhaled breath was measured sixteen hours after termination of exposure by connecting the animal through a rubber mask to an inhale-exhale valve.[33] The exhaled air was passed through a 150-ml gas collecting flask for a period of three minutes to obtain thorough flushing of the collector. The CS_2 in the exhaled breath was then measured by injecting 5 ml of diethylamine-copper reagent into the gas collector and allowing 30 minutes for the color developing reaction to become complete. The resulting color density was read in the Klett Colorimeter using a #42 filter, after the instrument was standardized against the reagent blank.

Throughout the period of exposure to 250 ppm of CS_2 no detectable quantities were found in the exhaled breath the following morning. CS_2 was found in the exhaled breath the morning after the first exposure to 500 ppm of CS_2, and throughout the period of exposure to this concentration. The mean value during this period was 1.4 ppm with a range of 1.2 to 2.4 ppm of CS_2 in the exhaled breath. Following the increase in exposure concentration to 750 ppm the exhaled breath concentration, measured 16 hours after exposure, increased to 3.1 ppm of CS_2 with a range of 1.2–6.0 ppm. The changes observed in exhaled breath concentration sampled in this way were proportional to the exposure concentration but did not indicate cumulation of the daily inhaled dose as the number of exposures increased. These data are interpreted as indicating that, in the limited range of concentrations used in this study, the CS_2 measured in the exhaled breath was not proportional to the CS_2 present in the body in combined form.

Hematocrit and Sedimentation Rate

The hematocrits and sedimentation rates (Westergren method) were determined on both the exposed and control groups of animals at the 10th, 24th, 32nd, and 34th weeks of exposure. The results are listed in Table VIII.

The values of the 32nd week were obtained one week prior to the first obvious signs of toxicity in four of the rabbits whose hematocrits were 35, 40, 43, and 45, respectively. It can be seen from the data in Table VIII that no significant difference existed between the two groups of animals.

TABLE VIII

Week	Sedimentation rate		Hematocrit	
	Exposed	Control	Exposed	Control
10th	1	1	41%	42%
24th	1	1	36%	39%
32nd	1	1	41%	41%
34th	1	1	42%	42%

Sedimentation rate = mm/hr.
Hematocrit = % red cells.

The sedimentation rates of all rabbits remained essentially the same throughout the entire period of exposure. Wintrobe[34] stated that the sedimentation rate is not controlled by the absolute concentration of the total plasma proteins or of the protein fractions, but that it depends on the relation of the various fractions to one another. These data substantiate the stability of the serum protein composition obtained by electrophoresis during the experimental period.

Electrocardiography

Changes in electrocardiograms have been reported by Lewey in patients and animals exposed to CS_2.[35] Brieger and his co-workers differed in their experimental findings and reported no significant changes.[36] The tracings provided by the animals in the present study were obtained from the precordial leads. Three positions were chosen: (1) V_1 was one cm to the right of the sternum, 3rd interspace from the xiphoid; (2) V_2 was one cm to the left of the sternum, 3rd interspace from the xiphoid; (3) V_3 was 1.5 cm to the left of the sternum, 4th interspace from the xiphoid. Tracings were obtained on all animals after signs of toxicity were observed. The only preparation necessary was clipping the fur from the desired anatomic areas. The rabbit was tied in the supine position to a restraining trough and allowed to become quiet before the tracings were taken. No anesthesia was used in any animal for these determinations.

The results obtained are in agreement with those of Brieger. In none of the tracings was there any sign of T-wave inversion or abnormality of the RST segment as reported by Lewey.[35] There were no abnormalities in the tracings of either the exposed group or the control group.

Urinalysis

Urinalyses were performed on both groups of animals at irregular intervals throughout the

period of exposure. The pH was always found to be on the alkaline side (pH 9) and the specific gravity was similar for the two groups of animals. At no time was any protein or glucose found in the urine of the exposed or control rabbits.

Clinical Observations

No clinical signs of toxicity, other than body weight effects, were observed during exposure to 250 ppm CS_2 for six hours per day, five days per week over a period of 16 weeks. In the 17th week the concentration was increased to 500 ppm CS_2 and maintained at this level for five weeks without positive clinical signs of CS_2 toxicity. At the 22nd week of exposure the concentration was increased to 750 ppm CS_2 and maintained at this level for 17 weeks at which time all animals exposed had developed clinical signs of toxicity and the exposure was terminated. At no time during the exposure were any signs of acute toxicity due to exposure observed. No behavior disturbances indicative of hyperirritability were observed in any of the rabbits. All rabbits remained easy to handle and submitted to the various procedures in the same manner as the animals in the control group during the entire study.

The earliest observation of obvious signs of toxicity developed in rabbit No. 7 in the 29th week. In the previous two weeks this rabbit ate only seven grams of food and had lost 1200 grams of body weight. The signs observed were loss of control of the hind legs to a point where the rear quarters were almost unable to support or move with the body. Examination of this animal after sacrifice for pathologic changes revealed that the refusal to eat was due to acute esophagitis.

During the 33rd week of exposure four additional rabbits displayed obvious signs of toxicity. These signs were uniformly partial loss of control over voluntary movement of the hind legs while movement of the rear quarters appeared to require great effort. While quiet, there appeared to be no loss of balance or alertness. However, the rabbits were notably unsteady while moving, indicating that coordination and control of the voluntary muscular systems were obviously impaired. By observation, the principal loss of muscular control in the rabbits seemed to be limited to the lower lumbar and rear quarters with practically no abnormality obvious in the movement of the forelegs, head or neck. The appearance of abnormal movement followed a decided loss in weight in each animal without exception.

In one animal, rabbit No. 21, in addition to the loss of rear quarters' muscular control a spastic contracture of the right facial musculature and loss of the right corneal reflex was observed. After the development of these signs rabbit No. 21 was removed from exposure and observed for six weeks before sacrificing. During this time the facial spasticity disappeared and the corneal reflex returned but no improvement in the control over the rear quarters was observed. A similar observation period in three other rabbits with only rear quarter impairment did not indicate any improvement of the condition within the period of observation. These observations are consistent with the degree of pathologic change observed in the brain and spinal cord and the copper content of these tissues.

All exposed animals had developed definite loss of muscular control in the rear legs by the end of the 38th week of exposure and exposure was terminated at the end of the 38th week.

The schedule for sacrificing the animals was adjusted so that half of the animals were observed during a post exposure period of four to six weeks while the other animals were sacrificed within one week after the termination of exposure.

In this experiment involving 16 weeks exposure to 250 ppm, five weeks at 500 ppm and 17 weeks at 750 ppm of CS_2 no complete paralysis of the rear quarters of any of the rabbits occurred. The signs of impairment observed indicate the development of an insidious and chronic toxicity to the voluntary control of the lumbar and rear quarters of the animal. The observation of neuropathologic lesions in the spinal cord of the rabbits exposed to CS_2 provides the reason for the signs observed. The sudden and continuous loss of body weight which began 6 to 14 weeks before the development of obvious signs of toxicity may be of prognostic value in regard to this type of toxicity.

Pathology of the CS_2 Lesion

The rabbits were sacrificed by means of intravenous injection of 3–4 cc Nembutal. Zenker-acetic acid was used as the fixative for all tissues except the nervous system. The brain and spinal cord were fixed in 10 per cent formalin, as were duplicate sections of internal organs for special staining. The tissues were embedded in paraffin. Routine sections were cut at 6 microns and stained with hematoxylin and eosin. In selected sections special studies were made by employing the following methods: trichrome stains, PAS, Weigert's basic fuchsin stain for elastic tissue,

Weil-Weigert's myelin sheath stain, Bodian's stain for axis-cylinders, gallocyanin stain for Nissl substance, Perls' iron stain and Scarlet R for fat.

Sections from the central nervous system routinely included the frontal, parietal and occipital lobes, basal ganglia, cerebellum and at least eight sections from various levels of the spinal cord. The cervical region of the spinal cord was not included because of technical difficulties experienced attempting to obtain it from the curvature of the neck. The optic nerves of a few rabbits were also examined.

Histologic examination of sections from abdominal and thoracic organs revealed no significant changes with but few exceptions.

The lungs were completely normal in most instances. In only a few exposed rabbits was acute or chronic pneumonia present. This, however, did not exceed the incidence of pneumonia observed in the control rabbits.

The heart in both groups frequently showed a minor degree of chronic interstitial myocarditis. In addition, two exposed rabbits had rather marked interstitial fibrosis of the ventricles with endocardial fibrous thickening. No coronary artery disease was observed in any animal. The thoracic and abdominal portions of the aorta were examined. The aortic arch was the only site of atheromatous plaques in both groups of animals in which they were present to a minimal and equal degree.

A few rabbits showed centrilobular congestion and mild fatty degeneration of the liver. This, however, was always associated with pulmonary disease and was evident in both groups of animals. No increase of hemosiderin was observed in the livers of any of the exposed rabbits.

In the spleen of exposed rabbits a very mild degree of hemosiderosis was present.

The kidneys of exposed rabbits showed an increased incidence of chronic interstitial nephritis when compared with control rabbits. This varied from a mild cellular infiltration to marked diffuse medullary fibrosis observed in two rabbits (Figure 7). In the fibrotic areas the tubules were absent or markedly atrophic, and the corresponding glomeruli were sclerotic. On gross examination the lesions were observed as multiple depressed areas on the kidney surface. Interstitial nephritis was also found in the control rabbits but in a milder form than that found in the exposed group.

Ten exposed rabbits showed varying degrees of cortical hyperplasia of the adrenals, and cortical adenomata were found in four animals. In the controls a mild degree of hyperplasia was present in one animal and two had small cortical adenomata. These conditions existing in the adrenals account for the greater weight of the adrenals in the exposed group which was discussed earlier in this report and substantiate the biochemical indications that the adrenals were hyperactive due to metabolic stress.

In no instance was any pathologic change found in the pancreas, thyroid or striated muscle from the thigh.

The central nervous system was the site of marked pathologic changes. This is in agreement with previous reports.[35, 37, 38]

The meninges of the brain showed pathologic changes in all exposed rabbits. These observations were noted for the first time in the two rabbits which were sacrificed after 12 weeks of exposure to 250 ppm CS_2. The changes consisted of meningeal swelling, proliferative thickening and diffuse infiltration with lymphocytes, and were observed in varying degrees in all lobes (Figure 8).

The cerebral cortex contained pathologic findings in all exposed rabbits. No definite localization of the lesions was observed. The pathologic changes appeared to involve individual nerve cells rather than definite regions of the brain. The cortical architecture was well preserved, but spongiosis was seen in a few animals. Some of the nerve cells showed degenerative changes that consisted of vacuole formation and fraying of the cytoplasm or shrinking of the entire cell with tortuosity of the dendrites. The nuclei of the nerve cells were often swollen, and the nuclear

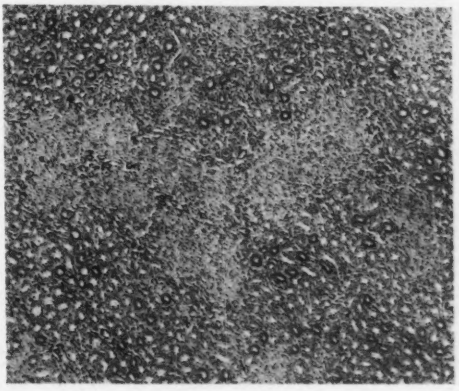


FIGURE 7. (15147 #15) Medullary portion of kidney from an exposed rabbit. Note the diffuse fibrosis and atrophy of tubules. (H & E stain, × 80)

membrane was indistinct. Satellitosis was usually mild, and only in a few instances was neuronophagia observed. No marked proliferation of the glia was observed. The small cortical blood vessels frequently showed mild thickening of the wall and proliferation of the endothelial lining (Figure 9). These changes were not found in all animals and varied greatly in extent and degree. In addition, perivascular infiltration was observed in four rabbits. No demyelination was demonstrated in the cerebrum of any exposed animal.

FIGURE 8. (15147 #9) Section of parietal lobe from an exposed rabbit. The meninges are thickened and diffusely infiltrated with lymphocytes. (H & E stain × 250)

Pathologic changes were observed in the cerebellum of each exposed rabbit. The most pronounced lesions involved the layer of the Purkinje cells. The latter were decreased in number, and degenerative changes were present in several of the remaining cells. In the pons of one exposed rabbit rather marked thickening of blood vessel walls was found.

The lesions in the spinal cord were the most striking pathologic findings in this study, and were found in all exposed rabbits at termination of exposure. The white matter of the spinal cord showed pronounced spongiosis which gave the involved areas a Swiss cheese-like appearance. Globular or stellate basophilic clumps were present in the spongy areas and stained black with Bodian's stain for axis-cylinders. These were interpreted as markedly swollen and degenerating axis-cylinders (Figures 10 and 11). There was marked loss of axis-cylinders, and in those that were sectioned longitudinally fragmentation was observed (Figures 12 and 13). No loss of myelin was demonstrated in the spinal cord of any animal. No fat was seen with Scarlet red in frozen sections. These observations are compatible with the possible inactivation of the coenzyme A dehydrogenase system, due to the unavailability of copper, as was found in the same tissue.

The lesions involved only the upper thoracic portion of the spinal cord. In only one rabbit could the pathologic changes be traced all the way down into the lumbosacral region of the cord. The most severe changes were in the anterior and lateral funiculi corresponding to the anatomical location of the anterior and lateral

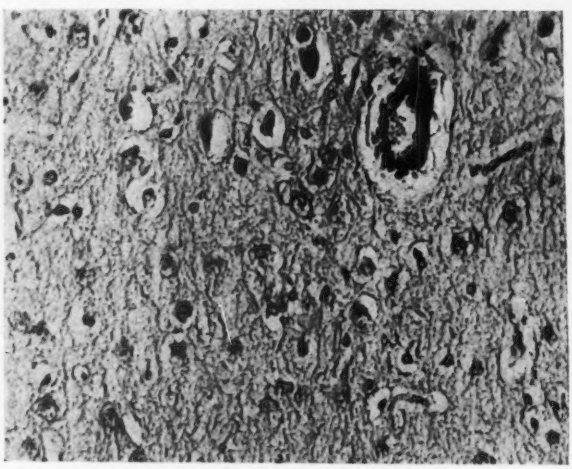


FIGURE 9. (15147 #8) Section of cerebral cortex from an exposed rabbit. The cortical blood vessels show thickening of walls and intimal proliferation. (H & E stain × 250)

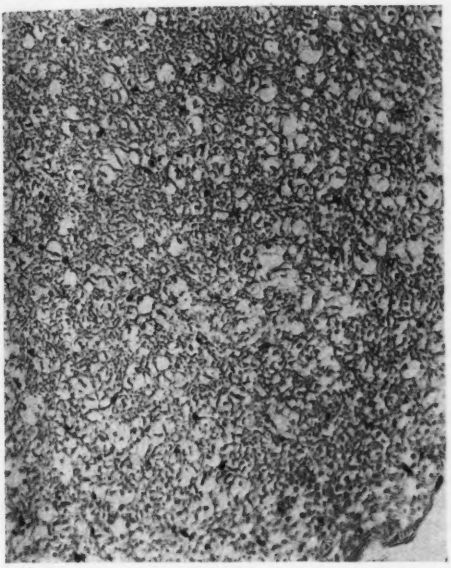


FIGURE 10. Section of anterior funiculus from upper thoracic spinal cord from a control rabbit. Compare this figure with Figure 11. (H & E stain × 160)

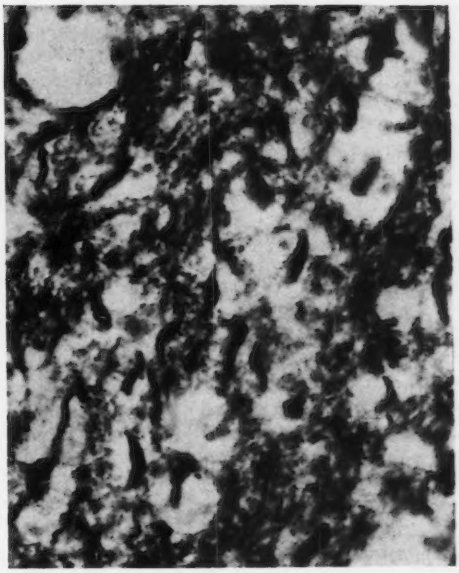


FIGURE 12. Axis-cylinder distribution in the spinal cord of a control rabbit. (Bodian stain × 650)

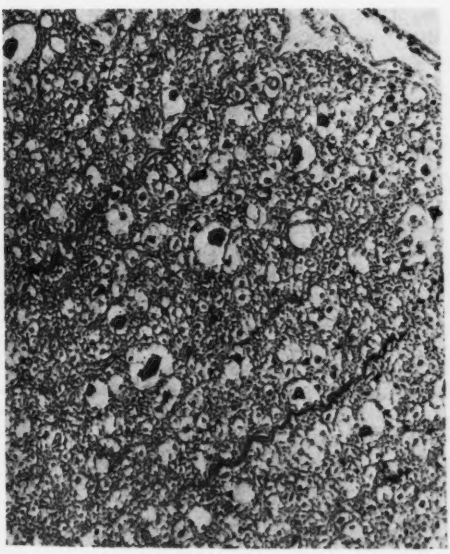


FIGURE 11. Area of anterior funiculus of spinal cord from upper thoracic region from an exposed rabbit. Note the spongy appearance and swollen axis-cylinders. (H & E stain, × 160)

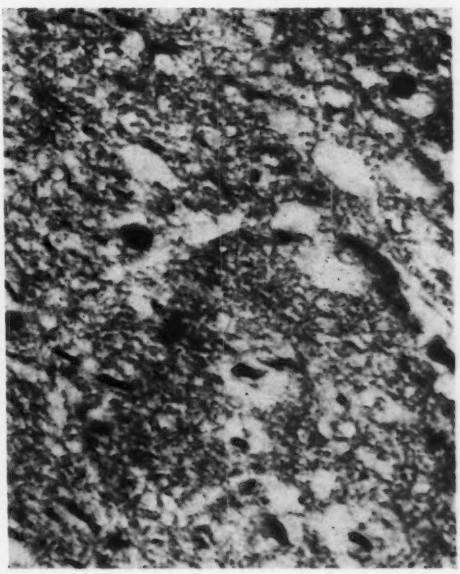


FIGURE 13. Section of spinal cord from an exposed rabbit. Note the paucity and fragmentation of axis-cylinders when compared with Figure 12. (Bodian stain, × 650)

corticospinal tracts. The posterior funiculus showed similar changes but to a lesser degree. In none of the six control rabbits were any changes of similar nature observed. The anterior horn cells were intact in all exposed animals. No vascular or meningeal changes were observed in the spinal cord.

No pathologic changes were found in the optic nerve.

Discussion

This study of CS_2 toxicity has been performed in rabbits by a series of consecutive inhalation exposures to three concentrations, 250, 500 and 750 ppm. The concentration of CS_2 was increased on each occasion after it was considered likely that disabling signs of toxicity would not occur in the near future. Although only suggestive signs of metabolic toxicity were observed during the exposures to 250 and 500 ppm it may be assumed that the changes produced during these exposures, although not disabling, hastened the onset of the disabling signs found during the exposure to 750 ppm of CS_2.

Through the work of Leonis[39] and Levy[4] the chemistry of the reaction of CS_2 with the NH_2 group of peptides and protein is fairly well understood. Buyle and Leonis[41] showed that, when the dithiocarbamate cyclized to form the 2-thio-5-thiazolidone, a characteristic spectrum with maximum absorption at 280 mμ was obtained. In the work of Chervenka and Wilcox[42] with chymotrypsinogen and its CS_2 derivative, a characteristic absorption spectrum was obtained in which maximum absorption bands at 250 and 292 mμ and a minimum absorption band at 272 mμ were obtained for the thiocarbamate form of the derivative.

The primary chemical reaction of the inhaled CS_2 with constituents of the body was identified by the development of ultraviolet absorption spectral changes in the serum from the exposed rabbits which were characteristic for thiocarbamate and thiazolidone groups. The known chelating effect of these changes in protein structure on the inorganic elements in the body was confirmed by a marked increase in zinc excreted in the urine collected during the first few weeks of exposure. Other changes observed, involving the beta lipoprotein, the magnesium activated fraction of serum alkaline phosphatase and wide fluctuation in serum zinc values, suggested that during the initial exposure to 250 ppm of CS_2 a metabolic disturbance involving the normal metabolic functioning of the body was taking place. These metabolic disturbances appear to be reflected in the failure of the animals to gain weight during the first 11 weeks of the study.

The ability of the exposed rabbits to adjust and compensate for the metabolic stress was shown by the gradual but steady weight gain observed from the 11th to the 24th week even though the level of exposure during this time had been increased to 500 ppm for the last five weeks.

Numerous references in the literature indicate that CS_2 acts as a metabolic toxin. Granati[43] and Lysina[44] report changes in serum proteins following CS_2 intoxication. Vigliani[1, 45] has reported that the beta/alpha lipoprotein ratio is increased in patients exposed to CS_2. However, stress, in the form of massive hemorrhage, has been shown to produce lipemia and atherosclerosis in rabbits. The production of atherosclerotic lesions in rats following the administration of ACTH has been reported by Wexler and Miller.[46] Young and Hayes[47] suggest that elevated levels of circulating epinephrine should be considered as a possible cause of chronically elevated serum, lipids and lipoproteins. Other stress phenomena, such as x-irradiation of chick embryo[48] and of the hind leg of a rabbit,[49] have also been shown to result in an increase of beta lipoprotein in the serum.

During the early phase of this study a consistent finding of elevated levels of beta lipoprotein in the serum has been interpreted as a stress response. Reference has been made to reports in the literature illustrating altered lipid metabolism in various forms of stress. The observation that the elevated beta lipoprotein found was not accompanied by an increase in cholesterol in the serum is an unusual characteristic in this type of change. As indicated in the report of Nath, et al.,[30] the 15 per cent level of protein may have effectively prevented the cholesterol rise in the pre-toxic stage of this work. However, during the later toxic stage of the experiment, when the animals were losing weight consistently, the cholesterol of the serum did increase, indicating that under the increased metabolic stress conditions caused by inhalation of 750 ppm of CS_2, the high protein content of the diet failed to protect the animals. This result suggests that the CS_2 can interfere with the proper utilization of dietary protein. This interpretation is consistent with the known chemical reactions between CS_2 and the amino groups of amino acids, although more direct proof of this point is needed.

During the toxic stage of the experiment another associated change taking place was the mobilization and utilization of body fat. Such a mobilization of the body fat stores indicates the serious nature of the metabolic disturbance present in the animal body as a result of inhaling

750 ppm of CS_2 and also accounts for the rise in serum cholesterol while the animals were losing weight.

Based upon the evidence obtained during the toxic phase of the experiment the effect of CS_2 on body metabolism may be outlined as follows: (1) inhaled CS_2 dissolves in the blood serum and slowly reacts with free amino groups of proteins and amino acids; (2) the thiocarbamate and thiazolidone groups thus formed chelate metal ions and interfere with the energy metabolism of cells as well as preventing the use of amino acids; (3) the metabolic stress calls forth an adrenal response with mobilization of the fat stores in an attempt to maintain the body metabolism; (4) the cells most vulnerable to the metabolic disturbance fail in their normal function and ultimately die.

The data obtained during the 17 week exposure to 750 ppm of CS_2 concerning the toxic signs confirmed, in part, the findings of previous authors[35, 37] on human and animal neuropathy caused by CS_2 exposure. The neurologic signs found in the rabbits were remarkably similar to those reported by Abe[38] in one human case of chronic CS_2 poisoning. In his case pyramidal signs and ataxia were present, but no sensibility disturbance or athetotic and chorea-like movements were found. Abe confirmed the clinical signs by pathologic findings after autopsy. Histologically the most severe damage was reported in the pyramidal tracts in the pons with marked degeneration of neurofibrils and myelin. Degenerative changes were found in the anterior and lateral funiculi of the spinal cord and in the Purkinje cells of the cerebellum.

The pathologic changes found in the exposed rabbits were similar to those found by Abe in his human case. The cerebral changes were comparatively mild and very little change was seen in the basal ganglia. Degeneration of loss of Purkinje cells and degenerative changes in the anterior and lateral funiculi of the upper thoracic spinal cord were found. These lesions did not show signs of regression during a period of four to six weeks observation without exposure.

The location of the lesions found in the spinal cord corresponded anatomically with the location of the pyramidal tracts. The pathologic changes observed in the cerebellum and spinal cord provide the reason for the neurologic signs of toxicity and the impaired control over the voluntary motion of the lower back and hind legs of the exposed rabbits. The observed difficulty in initiating and carrying out voluntary motion of the rear quarters and the lack of control over the abdominal and thoracic muscles, as shown by the respiratory studies, indicate failure of the lateral and anterior cortico-spinal tracts which are located in the lateral and anterior funiculi. Lewey's[35] observation of axis cylinder disintegration in the absence of demyelination was confirmed in this study. The lack of pathologic change found in the anterior horn cells in the anterior column of the spinal cord substantiates the absence of paralytic signs in the exposed rabbits.

The lesions found in the kidney of the exposed rabbits were classified as chronic interstitial nephritis, a disease commonly present in rabbits. The larger incidence and more severe form of this disease found in the exposed rabbits when compared with the controls indicates that CS_2 toxicity aggravates an already diseased kidney.

The indications of normal liver function found by electrophoretic examination of serum protein and maintenance of normal cholesterol and cholesterol esters was substantiated by histologic examination. Under the conditions of this experiment CS_2 did not display any of the activities characteristic of an hepatotoxin.

The observation of enlarged adrenals with cortical hyperplasia and adenomata in the exposed rabbits substantiates the general metabolic stress phenomenon postulated as a result of biochemical and physical data obtained. That such a metabolic stress can be compensated for, within limits, by the animal body is clearly shown by the body weight gain obtained after the 11th week of exposure. However, the limit of the ability of the body for metabolic compensation is also clearly demonstrated in this experiment by the series of disabling and injurious events which happened in the exposed rabbits shortly after increasing the exposure concentration to 750 ppm of CS_2. The first reliable sign of the toxic action of CS_2 found in these studies was continuous weight loss by the animals about two months before the onset of neurologic signs.

The observation of excessive amounts of zinc in tissues where no pathologic lesion was found clearly demonstrates the presence in these tissue cells of structures which will bind and hold the inorganic elements. These data substantiate those of the serum absorption spectra which identified the presence of thiocarbamate and thiazolidone groups in the serum. Further proof that the chelating activity of the protein derivatives are responsible for the toxic action of CS_2 is provided by the data on the copper content of the spinal cord. In those animals with spinal cord lesions which were sacrificed directly after exposure was terminated, the copper content of the spinal cord tissue was found to be low. In those animals which were removed from ex-

posure and observed for six to eight weeks before sacrifice, the copper content of the spinal cord was shown to have returned toward normal. Although copper is known to function as a structural part of cytochrome oxidase and coenzyme A dehydrogenase, too little data concerning the function of these enzymes in the metabolism of the rabbit spinal cord are available to do more than suggest that the chelation of copper and other inorganic ions by the thiocarbamate and thiazolidone groups present in the nerve tissue may be the cause of the lesions observed. This interpretation is supported by the observations of Soucek and his collaborators[50, 51] that brain tissue respiration and cytochrome oxidase are inhibited in vitro by CS_2.

Summary

The threshold exposure concentration which will injure rabbits maintained on a good diet has been shown to be between 500 and 750 ppm when administered six hours per day, five days a week. Early evidence that CS_2 combines with free amino groups in the body to form thiocarbamate and thiazolidone groups is presented. The sequestering action of these groups on the zinc and copper content of the tissues and their effect on metabolism is discussed.

The earliest toxic sign observed was loss of body weight which was followed in 6 to 14 weeks by loss of muscular control over voluntary movement of the lower back and rear quarters of the animal. The loss of muscular control was found to be due to lesions which developed in the spinal cord and brain tissue. These lesions were located in anatomical areas occupied by the pyramidal tracts of the spinal cord.

Based upon the evidence presented, the biochemical mechanism for the injury caused by these exposures to CS_2 may be outlined as follows:

1. Reaction of CS_2 with amino groups of amino acids and protein to form thiocarbamate which cyclizes to thiazolidone.
2. Chelation of polyvalent inorganic ions by the newly formed complexing groups to such an extent that cellular metabolism is disturbed.
3. The interference with metabolism causes a stress response and shift in metabolism which results in loss of body weight and indications that the fat stores of the body are being utilized.
4. Ultimately cell death and loss of associated function produce signs of injury.

The post exposure observations made indicate that little or no recovery of lost function was experienced during the period of observation although decided improvement in the tissue zinc and copper levels was observed.

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Effects on Growing Animals of a Continuous Exposure to Experienced Concentrations of Nitrogen Dioxide*

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Introduction

THIS STUDY was initiated to investigate the effects on animals of long term exposures to nitrogen dioxide in concentrations found in polluted urban atmospheres. By "effects" is meant any detectable biological or biochemical response of the experimental animals without reference to pathology.

Nitrogen dioxide (NO_2) is a universal component of polluted urban air. Vigdortschick[1] and his associates found a number of statistically significant differences including a reduction of blood catalase, in workers exposed occupationally to 2 to 2.5 ppm of NO_2. Gray[2, 3] and his coworkers have demonstrated that an exposure of rats to 9 to 14 ppm of NO_2 for 40 to 96 hours produced inflammation of the entire respiratory tract, but with exposures to concentrations of 4 ppm for four hours daily five days per week for six months they were unable to demonstrate any toxic effects.

Past investigations of the biological effects of NO_2 have utilized industrial or occupational air concentrations of the toxicant rather than urban air concentrations.

Procedure

Weanling Wistar rats were exposed continuously and dynamically to concentrations of NO_2 of 0.5 part per million and less. The 46 animals were fed Purina Laboratory Chow ad libitum, provided with tap water, and weighed periodically. Animals were removed from the exposure chambers approximately one hour each day for servicing. The animals were removed from the exposure system for a period of approximately three hours a day for one to three days when individual urine specimens were collected.

Groups of animals were sacrificed after exposures of 2, 4, 5, and 6 weeks. (The numbers in the groups were 8, 7, 11 and 20, respectively.) At the time of sacrifice, blood catalase concentrations were measured, sections of lung and liver tissue were preserved for microscopic examination, serum was separated and frozen, and the carcasses were frozen. The sera and the carcasses are being preserved for future analysis.

The animals, in cages, were placed in two chambers (one experimental, one control) which were one foot wide, one foot high, and three feet long. The bottom of each chamber was made of wood covered with teflon sheeting. The rest of the chamber was an open wooden frame covered on the inside with translucent teflon sheeting. Access to the interior was obtained by removing one end of the chamber which was held in place by bolts and wing nuts. An opening four inches in diameter in the removable end provided for incoming air. Air was removed through a half-inch exhaust opening at the opposite end. Room air, untreated, was the control air and was the base to which NO_2 was added to form the experimental air. Air was pulled through the system at the rate of two liters per minute by a Model 0211 Gast pump of the oiled type. Air temperature in the animal quarters was 23° to 26.5°C.

NO_2 was introduced into the experimental air by a small jet at the air intake opening to the experimental chamber. The pure gaseous NO_2-N_2O_4 (Matheson) was introduced daily into a series of 5 five-gallon bottles, 5 ml in the bottle nearest the chamber in the flow system, 6 ml in the second, 7 ml in the third, 8 ml in the fourth, and 9 ml in the last. Air passing through these bottles at a rate of 10 ml per minute carried the NO_2 to the entrance of the experimental chamber where it was introduced into the entering room air to form the experimental mixture. (Sampling of NO_2 in various areas of the chamber indicated good mixing so that it was not necessary to provide a baffle, as originally planned, to obtain more turbulence.) This system provided the animals with a constant dynamic exposure to NO_2 of 0.5 to 0.15 ppm. (Immediately after dosing the value rose to 0.7 ppm, dropped to

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0.5 after one half hour and 0.15 ppm at 23 hours.)

After the experiment was in progress it was discovered that the animal quarters were sprayed "as needed" to control insects. The spray consisted of piperonyl butoxide 0.8 per cent, and pyretheins 0.15 per cent in petroleum distillate 99.05 per cent and no record had been kept of its use.

Analytical Methods

Blood catalase was analyzed by the calorimetric method of Landahl[4] utilizing beef liver catalase from the Worthington Biochemical Corporation for the preparation of standards. Urinary creatinine was analyzed colorimetrically with alkaline sodium picrate by a modification of the assay of Bonsnes and Taussky.[5] Urinary glutamic acid and aspartic acid were analyzed with a one dimensional paper chromatographic method developed by Berry and Cain.[6]

NO_2 in the air of the chambers was sampled with 600-ml grab sample bottles and was analyzed according to the method described by Saltzman[7] modified in that n-naphthylethylenediamine dihydrochloride was utilized as the coupling agent instead of alpha-naphthylamine.

Results

Means for blood catalase values (Table I) were 342 units per milliliter of blood for the experimentals and 365 for the controls. The mean values at five weeks were 727 (experimental) and 600 (control), at six weeks the mean values were 269 (experimental) and 346 (control). The five- and six-week differences are statistically significant.

Glutamic acid (Table II) values are reported as micrograms per hundred micrograms of creatinine. The range of control values was 1.43 to 18.85 and the mean was 4.00 micrograms (μg) per 100 micrograms of creatinine. The mean experimental value was 6.74 μg (range 1.02 to 32.60). Aspartic acid (Table III) values are also reported as micrograms per 100 micrograms of creatinine. The mean control value was 2.75 μg (range 0 to 11.30). The mean experimental value was 5.31 μg (range 0.88 to 32.60).

The difference between control and experimental total glutamic acid values had no statistical significance. The aspartic acid means for the control and the experimental groups were significantly different statistically. Further breakdown of the aspartic acid data (time of exposure, sex, etc.) showed no significance. The great variation in individual values makes it unlikely that any breakdown which would reduce the sample size would show a statistically significant difference.

There was no difference in microscopic appearance of the lung and liver tissues of the two groups. The lungs of both showed symptoms suggestive of an endemic viral pneumonia.

No significant differences occurred in the weights of the two groups.

TABLE I

Blood Catalase Values (beef units/ml of blood)

	Time of exposure				
	2 weeks	4 weeks	5 weeks	6 weeks	Total
Experimental					
Mean	130	281	727	269	342
Range	53–193	140–404	667–843	193–404	53–843
Control					
Mean	180	205	600	346	365
Range	158–202	158–263	544–667	211–492	158–667

TABLE II

Glutamic Acid

(micrograms per 100 micrograms of creatinine)

Experimental	Mean	6.74
	Range	1.02–32.60
Control	Mean	4.00
	Range	1.43–18.85

TABLE III

Aspartic Acid

(micrograms per 100 micrograms of creatinine)

Experimental	Mean	5.31
	Range	0.88–32.60
Control	Mean	2.75
	Range	0–11.30

TABLE IV

Females	Day of birth*	Number of offspring	Age of mother at parturition days
Control			
1	1	11	63
2	2	4	64
3	3	8	65
4	5	3	67
5	—	none	—
Experimental			
1	1	10	63
2	9	7	71
3	14	9	76
4	14	6	76

* Numbering from the day of birth of the first litters.

Two incidental observations were made which may be of interest. Two of the sera from males which were exposed to NO_2 for six weeks were very milky, presumably with an excess of serum lipids.

The other incidental observation (Table IV) was made in the pilot study in which male and female rats were kept together. There were five control females and four experimental females all of the same age. Of these, one control female had no litter. The times of litters (numbering from the day of birth of the first litter) were: control litters on days 1, 2, 3, and 5, and experimental litters on days 1, 9, 14, and 14 (two litters on day 14).

Discussion

The differences obtained at five weeks and at six weeks between the mean experimental and mean control blood catalase values are both statistically significant. The surprising feature is that at five weeks the experimental mean was higher (727 units to 600) and at six weeks the control average was higher (control 346, experimental 269). There is a temptation to attribute this to uncontrolled variables, such as the spraying which occurred, but at present there is no valid explanation.

The large increase of urinary glutamic acid in the experimental animals has no statistical significance. It may be a true metabolic response, however, and will be investigated further. The increase in aspartic acid in the urine of the experimental animals over the controls was statistically significant. It is interesting that both glutamic and aspartic acids are involved in transanimation reactions, although this may have no relation to the observed increase in excretion by animals exposed to NO_2.

The delay in the births of litters to experimental female rats is interesting in light of the recent studies of Kotin.[8] Kotin and his associates observed a definite decrease in the ability of mice to conceive after a chronic exposure to air pollutants.

Differences between the control animals and the experimental animals may be attributed statistically to the presence of nitrogen dioxide in the experimental air, but the uncontrolled spraying with insecticide and the endemic pneumonia complicate interpretation of the data. Results obtained, while significant, may not relate to healthy animals in a simple air-NO_2 system. It is felt that further investigation along these lines is indicated. It is the opinion of the authors that observable effects have been demonstrated in animals exposed to experienced concentrations of a known air pollutant.

Summary and Conclusions

Weanling rats were exposed to 0.5 ppm of NO_2 in air. Suitable controls were maintained.

There was no significant difference in the microscopic appearance of lung or liver tissue.

Statistical significance can be attached to differences between the experimental and control groups in blood catalase values. The fact that at five weeks the experimental group was higher and at six weeks the control group was higher, however, makes interpretation entirely speculative at this time.

Glutamic and aspartic acid excretion increased in the animals exposed to NO_2. The aspartic acid increase was significant at the 5 per cent level; the glutamic acid increase was not.

Aside from conclusions regarding specific responses, it is the opinion of the authors that this study has demonstrated observable effects on experimental animals caused by an exposure to experienced concentrations of an air pollutant.

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Aerosol Filtration with Fibrous Media*

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THE MSA Company has specialized in aerosol filtration with fibrous media for more than 40 years because of its original and continued interest in the development, production and marketing of personal safety products of which respiratory protective equipment is a large segment. In expanding its markets and product lines, it was only natural that space filtration be given proper attention to take advantage of previously gained knowledge. Hence, a little more than eight years ago, a separate laboratory known as the Fluid Purification Equipment Laboratory was established to develop filters for gaseous and liquid streams for removal of specific gaseous, solid or liquid contaminants. An important product group in the overall program is that of space filters for aerosols.

Of great importance to this group are fibrous filtering media which exemplifies one of several techniques used to remove particulate matter from air or other gaseous streams.

The technical staff has evaluated more than 2500 different formulations of fibrous filtering media, many of which were developed in the MSA Laboratories. Seventeen of these have reached the production stage to this date. All forms of fibrous structures are routinely investigated—papers, cloths, pads, webs, and bats—and all methods of construction are considered—felting, weaving, wet accretion, air laying, flocking, fiber spraying, spinning and paper making. Many different types of fibers are involved in the investigations, such as mineral, ceramic, glass, metal, animal, plant, plastic and even carbon fibers. The task is a never-ending one.

To illustrate what can be accomplished by a program of this nature, and to support my belief that a realistically usable fibrous media can be developed for every aerosol filtering requirement, three completely different items out of the above noted large group have been selected for reporting.

Sprayed Plastic Webs

The first is a filter web made by spraying plastic fibers onto a moving screen with proper controls to regulate fiber diameter, length, distribution, apparent density and mat thickness. Many useful filter media can be made in this manner because of the control possibilities. Tight or loose webs, thick or thin, with any type of thermoplastic material readily liquified in a fast evaporating solvent can be made reproducibly. The one type in question appears as a thin (⅛-inch) web of medium weight (25 grams per square foot) made of Vinyon fibers, approximately three to four microns in diameter. Although many types of plastic media have been formulated, this one web has been selected because of its low resistance to air flow in combination with a relatively high dust retention efficiency and holding capacity.

For a high efficiency media, it exhibits an extremely low resistance to air flow, only 2 mm H_2O column at an air velocity of 28 feet per minute (Figure 1). By comparison, the well known 1106B medium for the Ultra Filter offers an air resistance approximately 50 times greater. Of course, the plastic medium does not prevent penetration of small particles as effectively as the 1106B, but it does have a reasonable degree of efficiency against 0.3 micron diameter dioctylphthlate (DOP) smoke (Figure 2), about 54 per cent at 28 feet per minute as compared to 99+ per cent for 1106B. This doesn't paint the entire picture, however, because the plastic web does have remarkable capture power for only slightly larger particles. For example, on the Bureau of Mines dust tests, it is over 99 per cent effective at the high speed of 28 feet per minute and on the NBS Atmospheric Air Dust Stain Test, it shows an efficiency of 83 per cent at this speed. At lower speeds, and it will undoubtedly be used as a low velocity medium, efficiency on the NBS test goes over the 99 per cent mark.

The loading characteristic is favorable also. On an artificial load test at 28 feet per minute, the web retains 6.38 grams of dirt per square foot before the resistance is increased from the original 2 mm H_2O column to 4 mm H_2O column (Figure 3). If an atmospheric air load will give the same results, it would mean that a 24 x 24 x 6-inch filter rated at 1000 SCFM could be made with an initial pressure drop of only 0.1 inch H_2O (about the same as the common furnace filter), and that it would hold more than a pound of dust before

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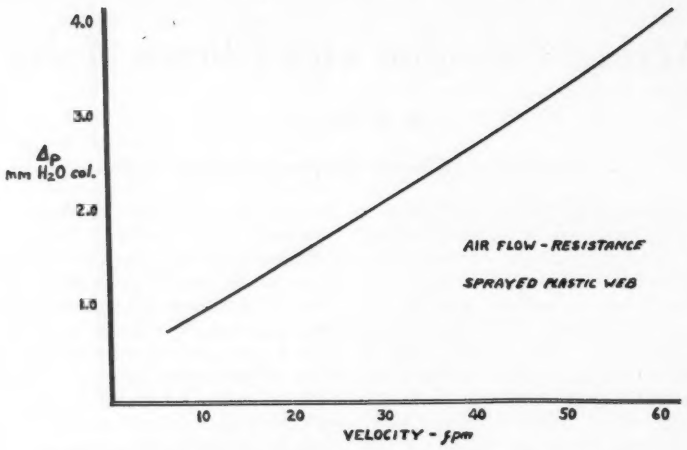

FIGURE 1. Air flow resistance of sprayed plastic web filter media.

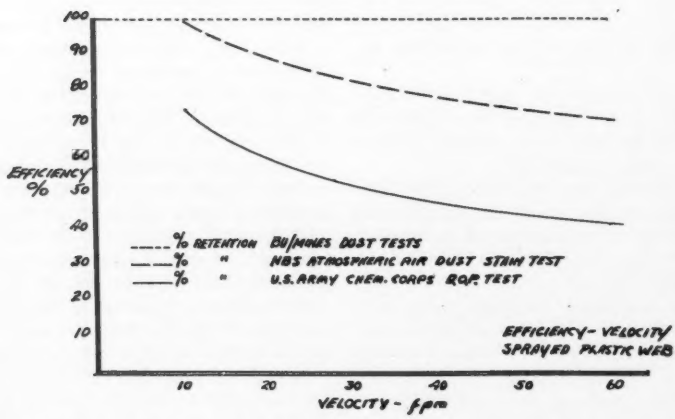

FIGURE 2. Filtering efficiency of sprayed plastic web at various velocities of air flow.

the pressure drop reached 0.2 inch of H_2O column. This filter would be over 99 per cent efficient.

Wet Accreted Pads

Turning to an entirely different approach in the design, development and production of air filtering media, let us look at the wet accretion method of laying and forming fibers. MSA has practiced this art for almost 20 years and it still proves to be one of the most practical ways to produce a final fluid filter. It combines the advantages of the paper making process with respect to fiber alignment with a technique which produces a final filter in one operation. Gas mask, respirator and automotive oil filters have been made by this process for a number of years.

The thought of combining aerosol and gas filtration in a single product has intrigued many of us over a long period of time. The U. S. Army Chemical Corps started research on this possibility over ten years ago and their latest combat mask incorporates filter pads containing a combination of fibers and finely divided treated charcoal, which remove harmful gaseous materials as well as particulate matter from air. MSA is experimenting with wet accreted pads of this type, and the one selected for this report also contains fibers and activated charcoal (Figure 4).

You will note that the MSA pad is extremely effective against small particles, being considerably over 99 per cent with 0.3 micron diameter DOP smoke, and that its carbon tetrachloride

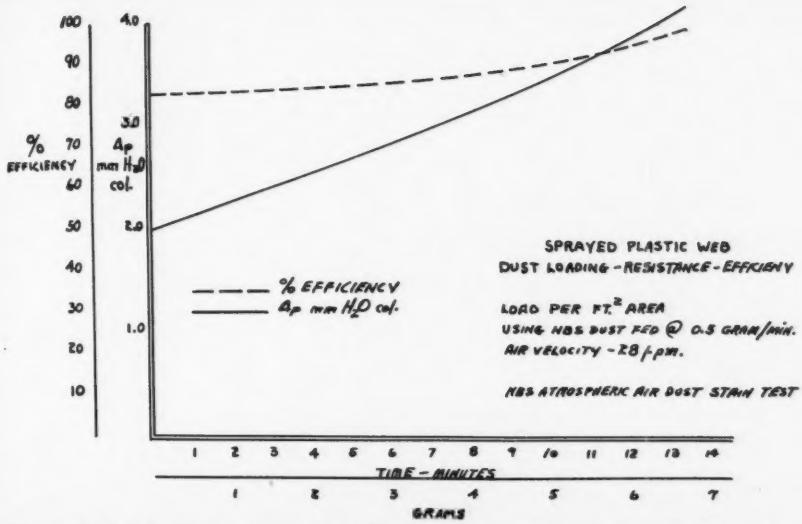

FIGURE 3. Dust loading, resistance and efficiency characteristics of sprayed plastic web.

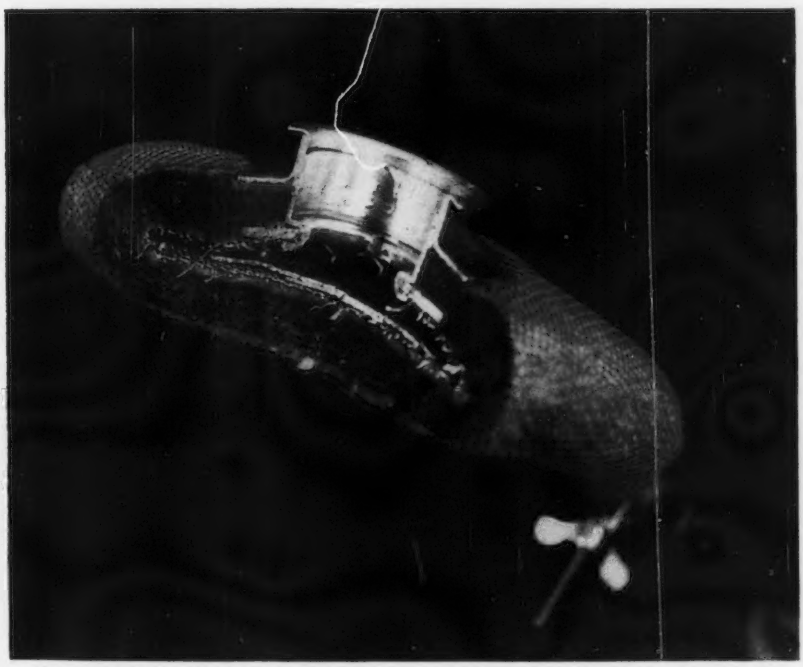

FIGURE 4. Cross sectional view of wet accreted pads of fiber and charcoal combination.

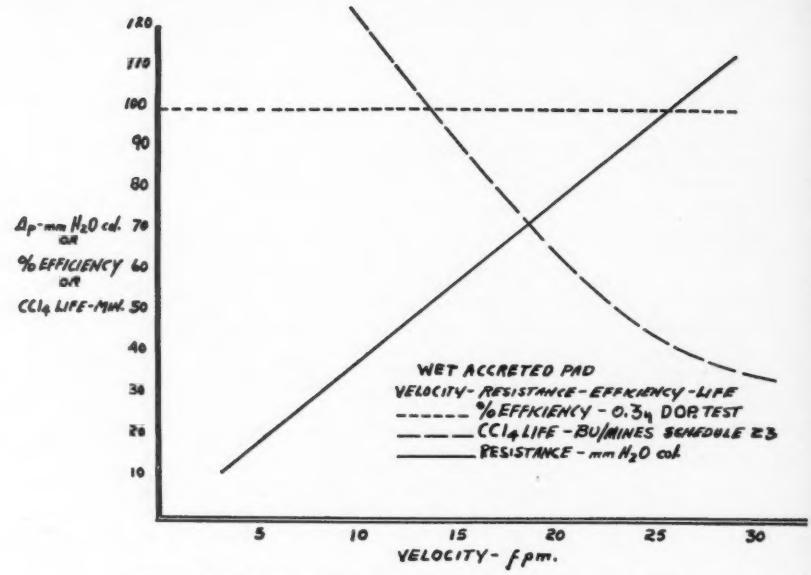

FIGURE 5. Performance characteristics of wet accreted pads.

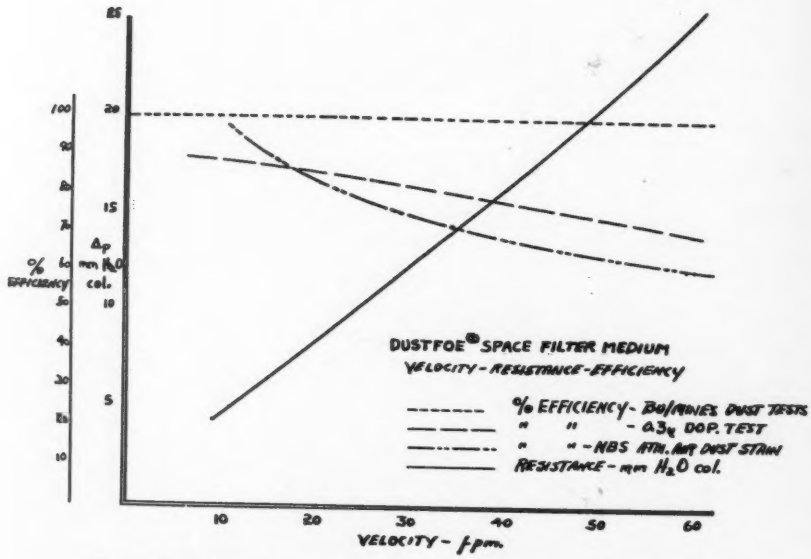

FIGURE 6. Performance characteristics of Dustfoe Space Filter medium.

(CCl_4) life is quite good (Figure 5). However, the resistance is high for respirator usage. A pad area in excess of 50 square inches would be required to perform effectively as a gas-particulate respirator. With this, one could expect an initial breathing resistance of about 30 mm H_2O (at 85 liter/min flow) and a gas life comparable to a Bureau of Mines approved organic vapor respira-

tor. Perhaps by decreasing the effectiveness against particulate matter and reducing the gas life, a reasonable compromise can be worked out.

Air Layed Fibers

The last example for this discussion is a media which has already been put to use in a product and in industry. It is made from glass micro-fibers which have been first air layed and then water compacted to affect a reasonably good fiber distribution and alignment. The resulting mat must be supported and for this reason is sandwiched between two pieces of fire-retardent scrim cloth. The initial resistance to air flow at a speed of 28 feet per minute is about 12 mm H_2O column or about one-eighth that of 11016B Ultra Filter medium. Efficiency against 0.3 micron diameter DOP smoke is about 84 per cent and it is over 99 per cent effective on the Bureau of Mines Dust Tests at this speed. On the NBS Atmospheric Air Dust Stain Test, a typical sample shows a 78 per cent efficiency at a speed of 28 feet per minute. Dust loading is good also, 4.25 grams per square foot at this same speed.

The filter material is, however, used in the Dustfoe Space Filter at a filter speed or an air velocity of only 12½ feet per minute. Hence, the pressure drop in use is quite low, typically 0.27 inch H_2O column initially (Figure 6). Efficiency on the NBS Atmospheric Air Dust Stain Test is for most cases in the 95 per cent region. The Dustfoe Space Filter will carry more than a one pound of atmospheric dust load before the pressure drop reaches one inch H_2O column. Eighty square feet of the filter medium is folded into a frame only 24 x 24 x 6 inches and this size will handle 1000 SCFM of air with a performance as stated above.

Our studies and these few examples lead us to one of the aerosol filtering axioms: present enough targets and/or create enough stream speed and tortuous paths within a fibrous media and then the liquid or solid particles will be separated from a gas stream and retained. True, there are a few other filtering mechanisms which must be considered from time to time, but if only these are considered, there are many possibilities in media design. There are so many, in fact, that we're quite certain a practical fibrous filter media can be developed for any aerosol filtering need.

New Spectrographic Standard

A NEW STANDARD SAMPLE of zinc spelter is now available from the National Bureau of Standards. Analyzed for sixteen elements the spelter standard is intended for checking and calibrating spectrochemical and chemical methods used in analyzing high-purity zinc spelter, and wrought zinc alloys. The spelter contains aluminum, iron, indium, copper, cadmium, manganese, chromium, tin, gallium, silicon, lead, magnesium, calcium, nickel, silver, and germanium in trace amounts. The concentrations of the first eight are certified, while the last eight are given but not certified. This standard and other similar standards available from the National Bureau of Standards can be of value to spectrographic laboratories in industrial hygiene for evaluating and improving their techniques of analysis of air-borne dusts and fumes from such materials.

Bar segments 1¾ inches square by ¾ inch thick may be obtained from the Standard Sample Clerk, National Bureau of Standards, Washington, D.C. for $10.00 per sample.

A Venturi Scrubber Installation for the Removal of Fission Products from Air*

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Introduction

THE CONSTRUCTION of a new radiochemistry building at the Los Alamos Scientific Laboratory made it possible to design an entirely new control and air-cleaning system for the gases released from the process of dissolving large filter papers containing fission products. In this process, a cellulose-type filter paper is dissolved in a hot mixture of nitric and perchloric acids. The off-gas is composed of perchloric acid mist, nitric acid mist, radioactive iodine vapor, mixed fission products, and oxides of nitrogen.

Two problems are associated with this effluent: Condensation of the perchloric acid in the collecting system and the evolution of radioactive materials. Under certain imperfectly understood conditions, the accumulation of perchloric acid in an exhaust system gives rise to a serious explosion hazard. For this reason, special hoods with water sprays are frequently specified for operations in which perchloric acid fumes are evolved. The release of the radioactive material, mostly in the form of iodine vapors, to the atmosphere is undesirable because of health considerations and because of the possible increase in the airborne activity of the building intake air. A slight increase in the activity of this air creates serious difficulties by raising the background count of the elaborate electronic equipment in the radiochemistry building. It was a basic decision, therefore, that the new building be provided with facilities for cleaning the exhaust air from the dissolving process.

After considerable study, the Radiochemistry Group decided that dry boxes and the small laboratory fume hoods used in the old building were undesirable from an operational standpoint. It was determined that a laboratory fume hood, eight feet long to permit the installation of two dissolving stations, would provide the most convenient setup for the operators. The required eight hoods of this type would exhaust approximately 16,000 cubic feet of air per minute (cfm), but cleaning this flow of air to the desired level of decontamination would require a large, expensive installation. Consideration was given, therefore, to small, local exhaust facilities located in close proximitiy to the source of contaminants and served by a separate exhaust system of approximately 350 cfm capacity. A typical hood installation is shown in Figure 1.

The estimate of the air flow to be cleaned was a basic factor in determining the most suitable type of air cleaning. Other considerations in the cleaning of this particular exhaust air, however, posed a number of problems. The perchloric acid mist cannot be allowed to condense in the duct work and must, therefore, be controlled or removed at the hood. The oxides of nitrogen and iodine exist as gases or vapors. In addition, since the dissolving process is performed at irregular intervals, it should be possible to activate and deactivate the air-cleaning system without adversely affecting its performance.

Initial consideration was given to wet filters for the removal of acid mists and to a scrubbing tower using silver salts for the removal of the iodine vapors. The experience at the Los Alamos Scientific Laboratory with wet filters,[1] however, has not been entirely satisfactory, and estimates of the initial and maintenance cost for the complete system, including a scrubbing tower, were rather high. Also, it was doubtful that this equipment would perform adequately under conditions of intermittent use.

The use of a venturi scrubber as a means of removing acid mists and small particulate matter appeared attractive. The main question in connection with this type of scrubber was its effectiveness in removing iodine vapors. A series of tests were, therefore, conducted on an existing venturi scrubber installation, and it was determined that the unit would not remove iodine vapors from the air stream if water was used as the scrubbing medium. A caustic solution was tried and removal efficiencies of about 95 per cent were obtained for a variety of iodine vapor loadings. The normality of the caustic solution did

* Presented at the Twentieth Annual Meeting of the American Industrial Hygiene Association, April 25–May 1, 1959, Chicago, Illinois. This work was performed under the auspices of the U. S. Atomic Energy Commission.

FIGURE 1. Over-all view of hood and two dissolving stations.

not appear to be an important factor and 1.0*N* sodium hydroxide was used in most experiments.

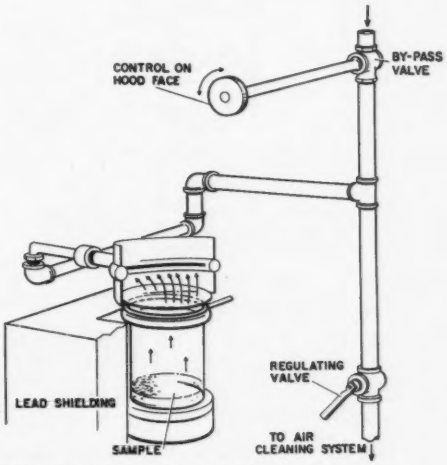
The use of the venturi scrubber with a caustic solution for this particular air-cleaning problem appeared to offer the following advantages:

1. A single unit would remove all contaminants of interest with good efficiency.
2. The scrubber would collect the contaminants in such a manner as to permit storage for radioactive decay and for ultimate disposal without exposing maintenance personnel.
3. The air collection and air cleaning systems could be thoroughly decontaminated at the end of an operating period, and minimum maintenance would ensure maximum efficiency for the next operation.
4. Because of the simplicity of the system, minimal maintenance would be expected. The ease of decontaminating the unit would, in any case, simplify and reduce the cost of necessary repairs.

Design Considerations

Local Exhaust Hood

The configuration of the local exhaust hood was determined by operating requirements, and the final design specified by the Radiochemistry Group is shown in Figure 2. Exhaust air requirements were determined empirically by varying the rate of exhaust while the dissolving operation was actually being performed. For the initial studies, the effectiveness of the air flow pattern was determined by observing the capture of the fog generated when dry ice was dropped in beakers of boiling water. The studies indicated that a flow rate of 20 cfm would give satisfactory control under conditions in which the evolution of the fumes was at maximum.



LOCAL EXHAUST HOOD & BY-PASS DETAILS

FIGURE 2.

TABLE I
Design Loading Factors

Material	Loading
Acid mist (nitric and perchloric)	500 to 3000 mg/m³
Total radioactivity	1 to 6 mc/m³
expressed as I^{131}	0.008 to 0.05 μg/m³
expressed as Sr^{90}	5 to 30 μg/m³
Solid particulate matter	<10 mg/m³
range of mass median size	0.58 to 11.0 μ

Venturi Scrubber

The design of the venturi scrubber was based on sixteen local exhaust hoods, exhausting 20 cfm each for a total flow rate of 320 cfm. At this flow rate, the anticipated loadings of interest are shown in Table I. Anticipated loadings are based on data obtained at the old dissolving installation and on the assumption that all dissolving stations would be operating.

The extremely low loadings and the existence of I^{131} in the vapor state were the major concerns of the Chemical Construction Corporation, fabricator of the venturi scrubber, in regard to the desired iodine removal efficiency of 95 per cent.

Final Design

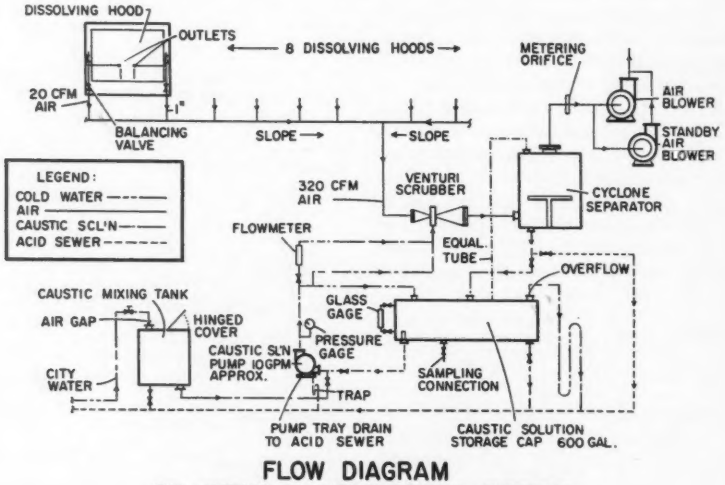
The final design of the entire system is shown in Figure 3 and is discussed in detail below.

Local Exhaust Hoods and Piping

The local exhaust hoods and piping were constructed of welded stainless steel. Horizontal runs of the piping were sloped, and regulating valves were placed only on vertical sections to facilitate drainage and washdown. A valve-controlled bypass was utilized to enable the operator to control the air flow through the hood and still maintain a constant flow through the venturi scrubber (see Figure 2).

Venturi Scrubber and Cyclone Separator

The venturi scrubber, approximately 48 inches in over-all length and with a throat diameter of 2⅜ inches, was also constructed of stainless steel. To provide for possible future needs, the venturi scrubber was actually designed by the Chemical Construction Corporation to handle 475 cfm of exhaust air with an expected pressure drop of 36 inches of water, but to be capable of operating at 320 cfm and 25 inches of water pressure drop with good air-cleaning efficiency. The throat velocities would be 15,420 and 10,390 feet per minute (fpm), while exhausting 475 and 320 cfm, respectively. It has been reported that throat velocities over 12,000 fpm are generally used in venturi scrubbers with pressure drops between 10 and 15 inches of water.[2] The design feed rate for the scrubbing solution, 1.0*N* sodium hydroxide, was established at six gallons per minute (gpm), at 15 pounds per square inch pressure (psi), with the system exhausting 320 cfm. This rate of approximately 19 gallons per 1000 cfm is higher than the reported rates of 2 to 9 gallons per 1000 cfm,[2] and accounts for the higher than usual pressure drop of 25 inches of water across the venturi scrubber.



FLOW DIAGRAM
COLLECTION & AIR CLEANING SYSTEMS

FIGURE 3.

The cyclone separator was not used as a cyclonic scrubber, although at one time such an arrangement was considered. A cyclonic scrubber in this system would have had a retention time of approximately 1 second, and it was thought that this factor would be important in absorbing the iodine vapors and oxides of nitrogen. The scrubbing solution, however, would have had to be fed to the cyclonic scrubber at a pressure of 100 psi. This would necessitate a separate pump or a high pressure system for both the venturi scrubber and cyclonic scrubber. It was decided, therefore, to convert the cyclone separator to a scrubber only if operating experience indicated it would be necessary.

Caustic Solution Recycling and Mixing Tanks

Since the installation was to be used on an intermittent basis, the capacity of the recycling tank (600 gallons) was designed to provide storage for sufficient caustic solution for the maximum anticipated run. The mixing and recycling facilities are essentially a standard installation for this type of equipment. A few special details, however, are the result of operating experience at the Los Alamos Scientific Laboratory. Leakage of radioactive liquids creates serious difficulties and consequently the pumps were mounted in trays equipped with proper drains, and a bypass was provided for the rotometer.

Exhaust Fans

Two exhaust fans (U. S. Hoffman Machinery Corporation, Model 4202, Type EBA), each with a rated capacity of 350 cfm at 50 inches of water, were specified for the installation. The extra fan was installed as a safety measure, and the electrical system so arranged that a failure of either fan automatically causes the other fan to operate.

Evaluation of Air-Cleaning System

The effectiveness of the air-cleaning system was determined by sampling upstream and downstream from the scrubbing units for total fission products, acid mists, and iodine vapors.

Acid Mists

The concentration of acid mists was determined by back titration of the caustic solution used as the collecting medium in two large impingers in series. A limited number of tests indicated that the air-cleaning efficiency of the unit for the combination of nitric acid mist and oxides of nitrogen was 90 per cent with peak loadings of 2 grams/m³. In the case of perchloric acid, with peak loadings of 3 grams/m³, removal efficiencies of 95 per cent were obtained. There is some mixing of the acids, but in the main the acid mists come off in two separate fractions. The efficiency for total acid mist removal for a complete run was approximately 92 per cent with a apparent average loading of one gram/m³.

TABLE II
Air-Cleaning Efficiency for Total Fission Products

Run	Dissolving stations operating	Loadings mc/m³	Air-cleaning efficiency %
1	6	0.3	94
2	5	0.1	95
3	4	0.05	94
4	4	0.01	91
5	6	0.4	96
6	4	0.15	92
7	3	0.05	93
			Ave. 94

Total Fission Products

Fission products were sampled from the air stream by means of a sampling train consisting of two large impingers in series followed by a high efficiency glass fiber filter paper. A caustic solution was used in the impingers. Aliquots of the collecting solution were evaporated to dryness on metal planchets. The radioactivity on the planchets and filter papers was determined by means of a gas flow proportional counter. The effectiveness of the system for total fission product removal is indicated by the results shown in Table II. The system was exhausting 320 cfm and the caustic solution was feed to the venturi scrubber at the rate of 6 gpm for all runs.

Radioiodine Vapors

Sampling for radioactive iodine was accomplished by a modification of a sampling train developed by Claude W. Still, AES, Idaho Falls, Idaho.[3] The train used in this study consisted of two high efficiency glass fiber filters, a Millipore

TABLE III
I^{131} Removal Efficiency

Run	Dissolving stations operating	Loadings		Removal efficiency %
		mc/m³	μg/m³	
1	4	0.03	2.4×10^{-4}	97
2	4	0.7	5.7×10^{-3}	98
3	4	0.9	7.3×10^{-3}	97
4	4	0.3	2.4×10^{-3}	96
5	6	0.05	4.0×10^{-4}	85
6	4	0.1	8.0×10^{-4}	93
7	3	0.03	2.4×10^{-4}	96
				Ave. 95

membrane filter, and two Willson organic vapor respirator cartridges. Provisions for extremely high filtration efficiency are necessary to prevent trapping particulate matter in the cartridges. The second glass fiber filter was provided as a safety feature in the event the first filter, which is damaged by the acid mists, ruptured completely. Tests indicated that 99 per cent of the iodine was collected in the first cartridge and no detectable iodine escaped through the second cartridge. Activity in the cartridges was determined by counting on a 2½ inch NAI crystal counter, and identified as I^{131} activity by determining the radiological half life.

The efficiency of the scrubbing unit for removing iodine is indicated by the results in Table III.

Summary

A local exhaust collection system and a venturi scrubber installation for the cleaning of exhaust air contaminated with acid mists and mixed fission products are described in detail. It was determined that 20 cfm exhausted by a local slot exhaust hood would control the maximum evolution of gases from a 1500 ml beaker. Features of the exhaust system that were designed to offset the hazard of perchloric acid condensation in the system included welded stainless steel construction, sloping horizontal runs, regulating valves only on vertical sections, and adaptability to cleaning by simple washdown.

The feasibility of a venturi scrubber with a caustic solution as the scrubbing medium for low loadings of iodine vapors (2.4×10^{-4} to 7.3×10^{-3} $\mu g/m^3$) was indicated by an average removal efficiency of 95 per cent.

Air-cleaning efficiencies for acid mists were dependent on the type of acid suspended in the air stream. Removal efficiencies of 90 per cent and 95 per cent were obtained with nitric acid and oxides of nitrogen loadings of 2 grams/m^3, and with perchloric acid loadings of 3 grams/m^3, respectively. Total fission product loadings ranging from 0.01 to 0.4 mc/m^3 were removed from the contaminated air with an average efficiency of 94 per cent.

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International Standards

THOUGH THE ANNOUNCEMENT of the establishment of an international pound and yard will have no significant effect on measurements in industrial hygiene, it is an interesting and encouraging step toward ultimate removal of the confusion caused by differing standards for units of similar or identical names. Standards laboratories of the United States, United Kingdom, Canada, New Zealand, Australia, and South Africa have agreed to adopt an international yard and an international pound to secure identical and precise measurements for science and technology. The international yard equals 0.9144 meter and the international pound equals 0.45359237 kilogram. Because of the substantial differences, agreement on an international gallon was not practicable.

The international inch is 2 ppm shorter than the U. S. inch and exactly equal to 25.4 millimeters. The international pound is about 1.5 ppm smaller than the U. S. pound.

Notes on Interferences by Oxides of Nitrogen with Estimations of Carbon Monoxide in Air by the NBS Indicating Tubes

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RECENTLY, this office was asked to determine whether the National Bureau of Standards carbon monoxide (CO) indicating tubes* were suitable for use in determining this gas in diesel fumes. These tubes, devised by Shepherd,[1] have been employed widely for some years as a convenient means of determining carbon monoxide in air. The tubes have definite advantages from the standpoint of cost, reliability and stability with age. However, serious limitations on the value of this method are the qualitatively known interferences of both nitric oxide (NO) and nitrogen dioxide (NO_2).[2] Concentrations of the mixed oxides as low as 30 ppm have been reported to bleach the color and yield erroneously low results.[3] Nitrogen oxides occur along with important sources of carbon monoxide. Typical exhaust gases from automobile engines contain 4 per cent CO and 0.06 per cent nitrogen oxides; typical diesel exhausts contain 0.1 per cent CO and 0.04 per cent nitrogen oxides;[4] and several hundredths of a per cent of nitrogen oxides also exist[5] in dynamite gases from tunnel blasting. In diesel exhaust and dynamite gases the relative proportions of nitrogen oxides have been reported[5] to be 35 per cent NO_2–65 per cent NO, and 52 per cent NO_2–48 per cent NO, respectively. These proportions change as the nitric oxide is gradually oxidized by air to nitrogen dioxide.

In this report the interferences of nitric oxide and nitrogen dioxide upon the determination of carbon monoxide were more precisely evaluated, and two methods for eliminating such interferences were devised and tested.

Experimental Method

Air at a flow of fifteen liters per minute was purified by scrubbing with dichromate in concentrated sulfuric acid solution followed by silica gel, then was blended with one per cent carbon monoxide in air, and eight per cent nitrogen dioxide in nitrogen, supplied from tanks. The nitrogen dioxide, carbon monoxide and air were each metered separately by glass rotameters. Check analyses were made for nitrogen dioxide with Saltzman's reagent.[6]

Known mixtures of carbon monoxide and nitric oxide were prepared in a dynamic system described more fully elsewhere.[7] One liter per minute of air, purified by passage through a universal gas mask canister, was blended with a mixture of the one per cent carbon monoxide and with one per cent nitric oxide in nitrogen from tanks. The latter was metered to the system by means of a motor-driven 50-ml glass hypodermic syringe. The apparatus was specially designed to dilute rapidly the nitric oxide before it could be oxidized to nitrogen dioxide and to provide a minimum flow-time above the sampling point. This oxidation proceeds rapidly at high nitric oxide concentrations, but quite slowly at low concentrations. Tests for nitrogen dioxide showed that only a negligible amount of oxidation was occurring in the system as operated.

All carbon monoxide analyses were made by the NBS field method, using a two-ounce rubber bulb with valve. Before sampling, time was allowed for the flow system to reach a steady state. Duplicate samples were taken in most cases, with identical results.

Interferences of Nitrogen Oxides and Their Elimination

Nitrogen dioxide was found to be a very serious interference in the determination of carbon monoxide, as shown in the upper section of Table I. As little as three ppm of nitrogen dioxide resulted in a 50 per cent error in the determination of 20 ppm of carbon monoxide, while seven ppm produced a similar error in the determination of 400 ppm of carbon monoxide. Thus, the NBS indicating tubes may be expected to give erroneously low results in the presence of nitrogen dioxide.

This difficulty was readily corrected by the insertion of an absorption tube to remove nitrogen dioxide ahead of the sampling tube. Ascarite®,

* Available commercially from Mine Safety Appliances Company and U. S. Safety Service Company.

TABLE I

Interferences of Oxides of Nitrogen in Carbon Monoxide Determinations by the NBS Indicating Tubes, and Elimination

Metered ppm			ppm CO read by NBS tube		
CO	NO_2	NO	Direct sample	Preceded by U-tube of Ascarite	Preceded by $KMnO_4$ & Ascarite
400	0	0	400		
400	7	0	200		
400	50	0	150		
400	95	0	100	400	
20	0	0	20		
20	3	0	10		
100	0	0	100		
100	0	7	100	100	
100	0	15	50	50*	100
100	0	65	10	10*	25
20	0	0	20		20
20	0	15	5		10

* Slightly darker than without Ascarite, but still reading closest to the color corresponding to the stated concentration.

a prepared absorbant of sodium hydroxide, has been reported[6] effective for this purpose, so a U-tube 100 mm high by 17 mm outside diameter was filled with Ascarite and used to absorb the nitrogen dioxide. (See Figure 1.) Table I shows that the interference of as much as 95 ppm of nitrogen dioxide was thus completely eliminated.

Nitric oxide was also an important, though less serious interference, as shown in the lower half of Table I. Absorption of nitric oxide is more difficult because of its low reactivity. The most practical method appeared to be its preliminary oxidation to nitrogen dioxide followed by absorption in that form. Such an oxidation was investigated by Thomas,[8] who reported that a simple bubbler containing 2.5 per cent potassium permanganate in 2.5 per cent sulfuric acid was effective. A dry method was felt to be preferable for field use. Accordingly, the same type of U-tube, as previously mentioned, was filled with a different packing in each leg, separated by a glass-wool plug. The upstream section contained potassium permanganate crystals; the downstream section was filled with Ascarite. The results obtained with this tube, as well as with a simple Ascarite tube, are given in Table I. These data indicate that the potassium permanganate-Ascarite tube was reasonably effective for eliminating interferences in gas mixtures likely to be found, although with high nitric oxide-carbon monoxide ratios the interference was not completely eliminated.

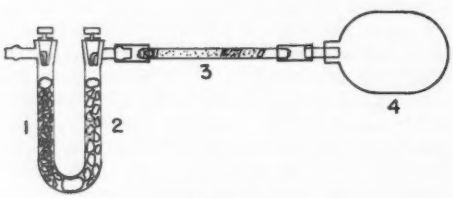

FIGURE 1

1. Solid potassium permanganate
2. Ascarite
3. NBS carbon monoxide tube
4. Squeeze bulb and valve assembly

Summary

Nitrogen dioxide and nitric oxide interference with carbon monoxide estimations by the NBS indicating tube were investigated. Both oxides were found to interfere seriously, the nitrogen dioxide to a greater extent. Nitrogen dioxide interference can be eliminated and nitric oxide interference considerably reduced by using a U-tube with solid potassium permanganate in the upstream leg and Ascarite in the downstream leg. The U-tube is flushed with the contaminated air, using the rubber bulb, and then connected ahead of the indicating tube. The field determination of carbon monoxide is then made in the usual way. No interference may be expected from concentrations of nitrogen oxides up to 15 per cent of that of carbon monoxide. If an NBS tube estimation without the U-tube does not give a color lighter than one made with the tube, then nitrogen dioxide can be presumed to be within its threshold limit[6] of five ppm.

The degree of interference eliminated by the tentative method reported here was sufficient for our immediate needs. Since a more extensive study is not planned at this time, this note is offered for the information of those who use the NBS carbon monoxide tubes.

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News of Local Sections

New Jersey Section

The June meeting was held at the Hotel Douglas in Newark, and twenty-nine members and guests were present. At this meeting the past presidents of the New Jersey Section were honored and presented with AIHA pins in appreciation of their services. Ten of the thirteen past presidents were present.

The feature address of the evening, *Fundamentals of Lighting and Seeing as They Relate to Industrial Environments*, was given by Frank Infanger of the General Electric Company. A telling point brought out in the talk was the fact that, under some conditions, advantages can be demonstrated for lighting levels significantly higher than those recommended by the Illuminating Engineering Society and considered to be "standard".

Southern California Section

The regular dinner meeting, with forty-nine present, was held May 14, 1959, at the Kaiser Steel Company in Fontana, California. Mr. Jack C. Rogers, President, opened the meeting and all members and their guests introduced themselves. A business meeting was held prior to a general tour of the plant. Louis Silverman and Ed Daniels discussed very briefly the highlights of the national meeting held in Chicago in April, especially with reference to certification.

Mr. Nuernberger spoke briefly on the role of safety in the plant. Dr. Hal Louis, Medical Director at Kaiser Steel, spoke of the occupational health hazards in the integrated mill, such as Kaiser Steel. Jack Smith, Air Pollution Control Director at Kaiser Steel, spoke on the progress of air pollution control at Kaiser Steel.

Metropolitan New York Section

A dinner meeting was held at the Medical Sciences Building at New York University-Bellevue Medical Center on May 21, 1959. Two papers were presented by members of the Division of Industrial Hygiene, New York State Labor Department: *Lead Hazard in a Plastics Plant; Its Evaluation and Control* by Dr. Robert Katz and Martin W. Jeremias, and *Three Unusual Industrial Hygiene Problems; Creosoted Wood Piling, Manufacturing and Testing Hydrogen Generators, and Handling Liquid Hydrogen Cyanide* by Martin W. Jeremias.

Upper Midwest Section

In its first year of operation the Upper Midwest Section has been quite busy with meetings and growth. From a total charter member group of 20 we now have 31 members with many prospects. The membership is fairly evenly divided among nurses, doctors and scientists as well as governmental, academic and industrial representatives.

At the fall dinner meeting Mr. Ken Nelson was the guest speaker. He discussed industrial hygiene problems of the smelting industry and also gave everyone in attendance a good picture of the AIHA organization.

In February, our speaker was Mr. Hibbert Hill, Chief Engineer of the Northern States Power Company which serves Minneapolis and St. Paul. Northern States Power is building a nuclear reactor for the development of power in Sioux Falls, South Dakota. The topic of the speaker was concerned with the industrial health problems during the operation of reactors.

On March 10, Minnesota Mining and Manufacturing Company of St. Paul was our host with Dr. John Ryan, Chief of the Radiochemical Research Division, discussing *Research in Radiochemistry Conducted by the 3M Company.* Following his interesting talk a tour of their facilities at the Central Research Laboratory in St. Paul was made.

On April 22 another topic in our occupational health field was covered by Dr. Leonard Schu-

man, Chief Epidemiologist of the School of Public Health, University of Minnesota. He discussed epidemiological aspects of air pollution.

Our meetings have all been well attended relative to the total membership. In the May meeting the newly elected officers of the section will be installed. These officers include: Mr. Ralph Wands, President, Dr. H. J. Paulus, President-Elect, Mr. George J. Raschka, Secretary-Treasurer, and Miss Bernadine Giuliani, Director for a two year term.

Northeastern Michigan Section

On May 13, 1959, at a regular meeting of the Northeastern Michigan Section at the Dow Chemical Company, Midland, Michigan, the following officers were elected: President, Mr. Mark Wolf, Dow Chemical Company; Secretary-Treasurer, Mr. Vic Troyer, Michigan Mutual Liability Company, Saginaw; and Director for a three year term, Mr. Arthur Rowe, Michigan Department of Labor, Saginaw.

Rocky Mountain Section

This Section periodically puts out a Newsletter with pertinent news of personnel. Paul Urone and Carl Jensen have been receiving publicity in the newspapers for their work in air pollution and radiation controls respectively.

The annual fall get-together of the Section will be held October 16 and 17 at Albuquerque, New Mexico. The program will include a plant tour, a dinner, technical papers, and the business meeting. By way of extra dividend will be lots of corridor sessions and good fellowship.

Personnel Notes

The University of North Carolina announces the appointment of Dr. Fritz Sulzer as Assistant Professor of Sanitary Sciences, Department of Sanitary Engineering, School of Public Health at Chapel Hill.

Dr. E. Gifford Upjohn, President of The Upjohn Company, and George A. Jacoby, Director of Personnel Relations for General Motors Corporation have been named to the Board of Governors of the Institute of Industrial Health, University of Michigan. They succeed retiring members, Michael Gorman and Dr. Max Burnell.